Setting priorities in CNF particle size measurement: What is needed vs. what is feasible

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ABSTRACT: Measuring the size of cellulose nanomaterials can be challenging, especially in the case of branched and entangled cellulose nanofibrils (CNFs). The International Organization for Standardization, Technical Committee 6, Task Group 1—Cellulosic Nanomaterials, is exploring opportunities to develop standard methods for the measurement of CNF particle size and particle size distribution. This paper presents a summary of the available measuring techniques, responses from a survey on the measurement needs of CNF companies and researchers, and outcomes from an international workshop on cellulose nanofibril measurement and standardization.

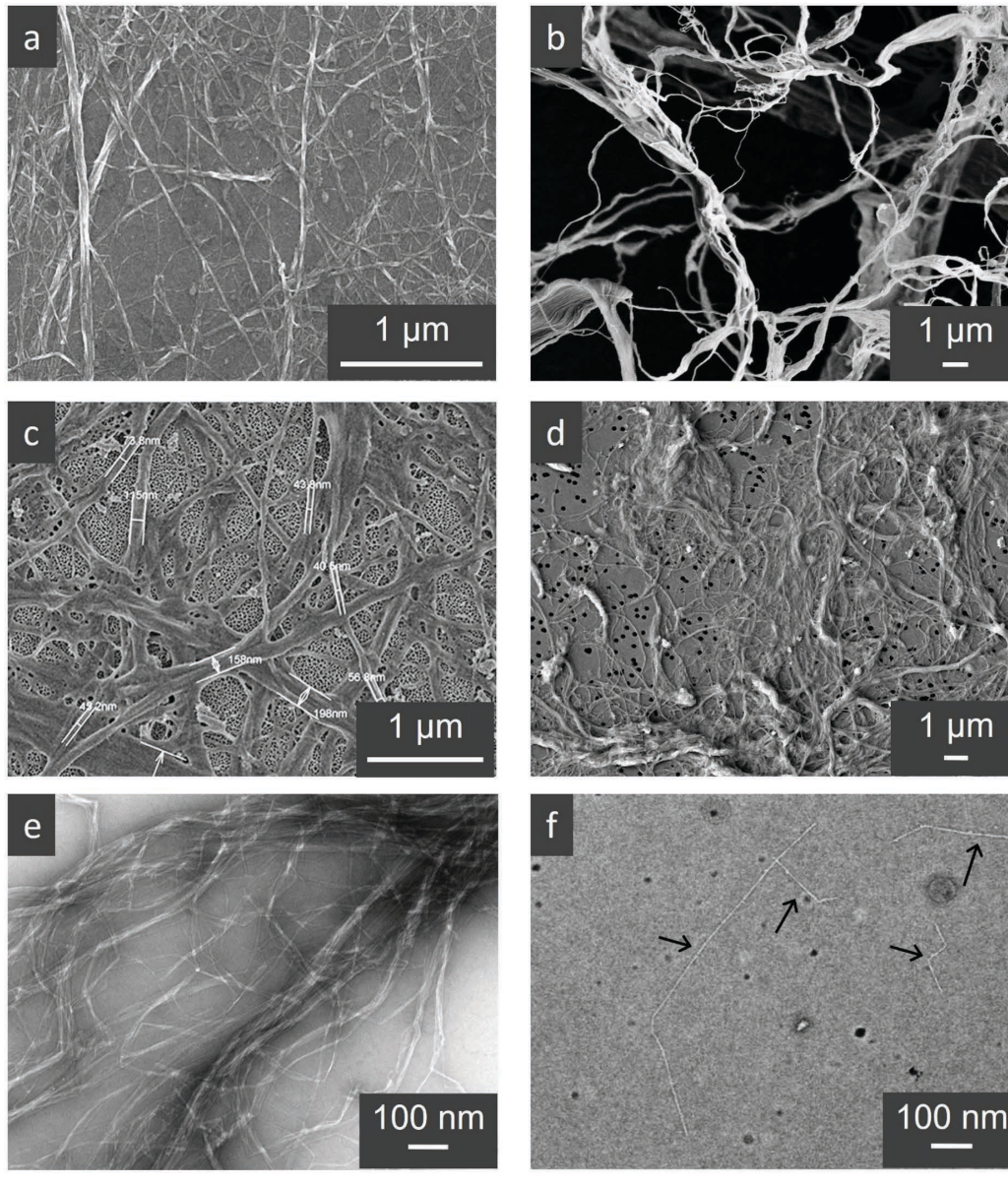
Standardization needs differed among groups, with Japanese companies mostly requiring measurements for product specification and production control, and other companies mostly needing measurements for safety/regulatory purposes and for grade definitions in patents. Among all the companies, average length and width with percentiles (D(10), D(50), D(90)) were the most desired measurands. Workshop participants concurred that defining the location(s) on the CNF at which to measure the width and the length is an urgent and complex question. They also agreed that methods are needed for rapid particle size measurement at the nanoscale.

Our recommendation within ISO is to start work to revise the definition of CNFs and develop sample preparation and measurement guidelines. It was also recommended that further research be done to reproducibly prepare hierarchical branched CNF structures and characterize them, develop automated image analysis for hierarchical branched CNF structures, and develop a classification system encompassing measurements at multiple size ranges from micro- to nanoscale to fully characterize and distinguish CNF samples.

Application: The theme of this research paper is "Addressing the gap: What is needed vs. what is feasible" in CNF particle size measurements. Readers will learn about the challenges in particle size measurements; why such measurements are needed in CNF production control, product specification, grade specification, benchmarking, and safety/regulatory requirements; which measurement techniques show promise; and a pathway for future efforts in standards development to address the measurement challenges.

Cellulose nanomaterials (CNMs) are fibril-like cellulose particles, with dimensions of 3–100+ nm wide by 50–2000+ nm long, that exhibit unique properties and functionalities distinct from molecular cellulose and wood pulps due to their size, morphology and large surface area [1, 2]. They are inspiring advances in cellulose science, technology, and product development in an ever-growing application space. The different types of CNMs present a wide spectrum of particle sizes, morphologies, and surface chemistries that control their properties, performance, and safety [3]. Thus, when working with CNMs, it is critical to have a comprehensive and accurate characterization of these features. Two broad classifications of CNMs have been defined: cellulose nanocrystals (CNCs) and cellulose nanofibrils (CNFs). The CNCs are rod-like particles mainly produced by concentrated acid hydrolysis; they are nanoscale in both length and width, with aspect ratios of less than 50, having tapered ends, no branching, and showing no entanglement. Approaches for the measurement of CNC particle length, width, and height have been extensively developed: ISO has developed a technical specification ISO/TS 23151:2021 "Nanotechnologies—Particle size distribution for cellulose nanocrystals," including interlaboratory comparisons of dimensions obtained using transmission electron microscopy (TEM) [4] and atomic force microscopy (AFM) [5].

In contrast, CNFs are fibril-like nanofibers composed of at least one elementary fibril containing crystalline, paracrystalline, and amorphous regions, with aspect ratio usually greater than 10, which may contain longitudinal splits, entanglement between particles, or network-like structures as defined in ISO/TS 20477:2017 "Nanotechnologies—Standard terms and their definitions for cellulose nanomaterial." This essentially covers all the CNMs produced by mechanical treatment of plant materials that are often combined with chemical or enzymatic pre-treatment steps and denoted by industry and academia as "nano- or microfibrillated cellulose" (NFC or MFC, respectively), and "cellulose



1. Typical images of entangled cellulose nanofibrils (eCNFs) and individualized cellulose nanofibrils (iCNFs). Scanning electron microscope (SEM) images of: a) bleached eucalyptus kraft pulp processed for 50,000 revolutions in a PFI mill and then passed three times through a homogenizer at a pressure of 1000 bar; b) University of Maine CNF material produced by disk refining; and c) fine fraction of Borregard microfibrillated cellulose (MFC) [41]; d) FibreLean coarse MFC. Transmission electron microscopy (TEM) images of: e) eucalyptus kraft pulp fibrillated via SuperMassColloider at 1500 rpm [12], image provided by co-authors from the National Institutes of Health (NIH); and f) iCNF, showing individual particle fibril morphology, with fine cross-section diameter and minimal branching [6], image provided by co-authors.

nano- or microfibrils" or "cellulose nano- or microfibers" (CNF or CMF, respectively).

The microscopy images presented in **Fig. 1** show examples of CNFs prepared by mechanical means from a number of different sources, including by commercial producers. The difficulties of measuring typical CNF materials are clear; there is a wide distribution of fibril diameter, and it is unclear in many cases whether they are fully separated or have become entangled during the process of preparing the sample for imaging. While fibril and branching diameters can be directly measured using imaging techniques, lengths cannot, as it is generally impossible to identify the start and end of individual fibrils. In this review report, CNF materials were differentiated into two broad categories: entangled cellulose nanofibrils (eCNFs) as shown in Figs. 1a, 1b, 1c, 1d, and 1e and individualized cellulose nanofibrils (iCNFs) as shown in Fig. 1f. Whereas eCNFs are typical CNF materials (e.g., NFC, MFC, CMF, etc.) that are very often

entangled with each other to form a complex fibrous network, iCNFs are discrete cellulose nanofibrils with minimal or no branching. Unfortunately, the lack of standard methods for particle size characterization have hampered efforts at optimizing CNF production, quality control, and use in various applications.

To provide a framework for the characterization of eCNF morphology, the ISO Technical Committee (TC 6—Paper, board and pulps) through a Task Group (TG 1—Cellulosic Nanomaterials) is exploring opportunities to develop standards for the measurement of eCNF particle size and particle size distribution. To achieve this, information regarding standardization needs and priorities for particle size measurements of eCNFs was gathered in a two-part process. First, an online survey was distributed to international CNF producers, end-users, and researchers working with these materials. Following this, a virtual workshop "Workshop on cellulose nanofibril particle size measurement: Setting priorities in standard development" was co-hosted by ISO/TC 6/TG 1 and TAPPI's Nanotechnology Division on September 14-15, 2021. The workshop provided an active dialogue to gain a broad perspective from the global community on: i) the capabilities and limitations of measurement methods already being used, ii) the feasibility of these methods for implementation in industry, iii) CNF features to measure for development as standard methods, and iv) the identification of priorities in standards development needs.

This report provides a general context as to why particle size measurements are needed for eCNFs, the difficulties and challenges of eCNF particle size measurement, and a summary of the findings from the survey and the workshop. The theme of this report is "Addressing the gap: What is needed vs. what is feasible"; priorities for future efforts in eCNF particle size measurement standards development that address what is needed and what is feasible will be presented.

MEASUREMENT APPLICATIONS

Standard CNF particle size determination methods that characterize length and width, along with their distributions, are needed to support CNF *production control and product specification*. The length and width of CNFs determine their physical properties (degree of entanglement, flexibility, flow properties in suspension), chemical properties (surface area available for modification), and optical properties (transparency in suspension and film forms). All these properties must be controlled at all stages of production and conversion to tailor the properties of the final product, to ensure a reproducible product, and to achieve reliable performance metrics for specific applications. Accurate and repeatable measurements of particle size and particle size distribution (PSD) are also critical to selecting appropriate processing equipment and conditions, thereby avoiding equipment malfunctions and allowing production of CNFs with the target size and properties.

Particle size measurement techniques are also needed to compare different CNF products; standard measurement methods for CNF particle size determination will ensure consistency in testing, quality control, *benchmarking*, and materials specifications, at both the fundamental research and global production levels. Compliance with *safety/regulatory requirements*, such as the EU definition of "nanomaterial," will rely on data obtained using standard methods to measure and count CNFs. Comparable particle size testing methods and results will also be vital to *grade specification* for the granting of patents. Ultimately, standard CNF particle size measurement methods will provide essential support for the commercialization of CNFs by facilitating international trade, harmonizing the implementation of regulations, and allowing differentiation among competitive products.

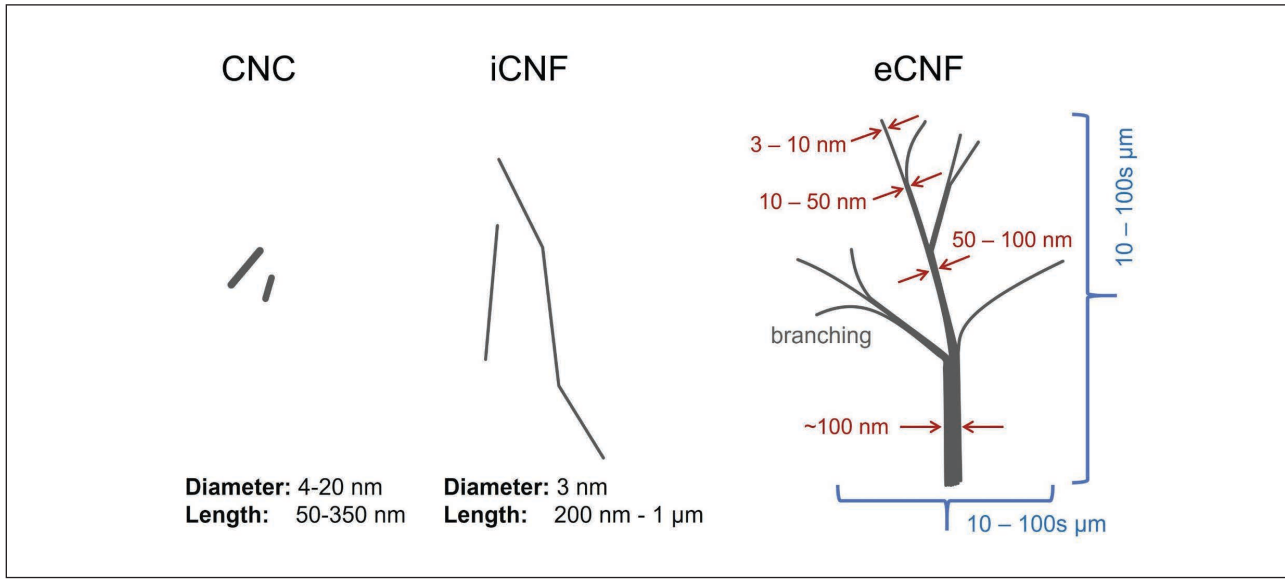
CHALLENGES OF CNF PARTICLE SIZE MANAGEMENT

Although our industry consultation via survey revealed that particle size and particle size distribution (PSD) measurement was a top priority for CNF producers, standard particle size measurement methods for CNFs are not yet under development. There are several practical reasons why such standards do not already exist, which are primarily associated with the complex particle morphology of CNFs. **Figure 2** shows a generalized representation of the particle morphology for CNCs, iCNFs, and eCNFs. Measurement challenges inherent to iCNFs and eCNFs are described in the following sections.

Individualized cellulose nanofibrils

Individualized cellulose nanofibrils (Fig. 1f and Fig. 2) are defined in ISO/TS 21346:2021 "Nanotechnologies—Characterization of individualized cellulose nanofibril samples" as a discrete cellulose nanofibril composed of one elementary fibril with ionic functional groups on its surface. Most commonly, iCNFs can be produced using a TEMPO-mediated oxidation pre-treatment of the cellulosic material to impart anionic carboxylate surface groups that repel each other and allow defibrillation (while also depolymerizing the cellulose). The iCNFs have a fibril morphology, with uniform cross-sectional diameters of around 3 nm, lengths ranging from hundreds of nanometers to several micrometers, and no branching; they are essentially elementary fibrils. The iCNF morphology as shown in Fig. 1f is dramatically different from that of eCNF, as shown in Figs. 1a, 1b, 1c, 1d, and 1e. Given the more homogeneous nature of iCNFs, obtaining representative samples for measurements is possible; however, several challenges remain:

- The acicular morphology of iCNFs makes interpreting light scattering data difficult.
- The iCNFs are too small to be detected optically.
- The iCNFs are too small to be detected by most scanning electron microscopes (SEMs), requiring AFM or



2. Schematics of cellulose nanocrystal (CNC), iCNF, and eCNF illustrating their generalized particle morphology. The CNCs are rod-like and iCNFs are fibril-like; both can be described by two parameters (diameter and length). In contrast, eCNFs are complex hierarchically fibrillated "branched" objects.

TEM techniques.

- Dispersion behavior under different conditions (e.g., concentration, pH, ionic strength) can be hindered by the surface charges on iCNFs; for example, iCNFs form gels at much lower concentrations, which may prevent the use of some measurement techniques.
- The iCNFs typically have kinks along their length (Fig. 1f), increasing the complexity of measurement by image analysis [6,7].

Entangled cellulose nanofibrils

Entangled cellulose nanofibrils are broadly defined in this report to represent the cellulose nanofibrils produced primarily by mechanical means. It is unclear how to best describe eCNF morphology, as it can vary quite extensively. The SEM images in Figs. 1a, 1b, 1c, and 1d show a wide distribution of fibril diameters and the presence of a network-like structure, and the TEM image in Fig. 1e more clearly shows the nano-sized fibril features, the bundling of fibrils, and nanoscale network-like structures. The simplified schematic representation of an eCNF particle shown in Fig. 2 does not represent all types of morphologies observed; rather, it highlights some of the typical features that make the size of these particles so difficult to measure. The overall object can be tens to hundreds of micrometers in size in all three dimensions and consists of hierarchically branched structures of nanosized fibril structures having diameters ranging from 3 nm to 100 nm and length segments of tens of nanometers to micrometers in length. The eCNFs are very often entangled with each other to form a complex fibrous network. As such, eCNF lengths and widths are difficult to define and measure:

- Although eCNF suspensions are a collection of macrosized, microsized, and nanosized objects, the total surface area of which can be directly related to the degree of fibrillation of the parent fibers, it is challenging to define the nanofibril length itself for measurement purposes.
- The macro- and micro-sized fibrils in eCNF samples can be measured using fiber analyzers that are designed for pulp fiber measurement and can typically detect fiber widths in the range of tens of microns and fiber lengths from 100 µm to several mm; however, it is unclear what their number fraction or mass fraction of such particles are within a given eCNF sample.
- Entangled networks of CNFs prevent the detection of separate or individual nanofibrils by microscopy or light scattering techniques, as the inherent intertwining of the nanofibrils makes it difficult to physically separate them and to define the beginning and ending of each nanofibril. This is an acknowledged problem, particularly for length measurement [8].
- The high aspect ratio of eCNFs can prevent both dimensions from being measured simultaneously by microscopy and other techniques.
- The very irregular morphology of eCNFs makes the data obtained by some indirect techniques, such as light scattering, more complex to model and interpret, especially as most standard models assume spherical particle shape.
- The considerable polydispersity of eCNF morphology also leads to difficulty in obtaining accurate measurement statistics [9]. Width often varies along the nanofibrils and across the hierarchical levels of branching (Fig. 2).
- Sample preparation often requires dilution and fraction-

ation and can lead to sub-samples that are almost certainly not representative of the material being tested. For microscopy and other techniques, high nanofibril counts and many parallel tests/images are therefore required for good statistics, greatly increasing the workload.

- The eCNFs exhibit different properties and behavior depending on their environment; different challenges can therefore predominate, depending on whether eCNFs are measured in the dry or wet (in suspension) state. In the wet state, it is possible for the eCNF structure to "open" up, while in the dry state, the eCNF structure can collapse on itself (e.g., bundling, agglomerating). Ideally, the testing or measuring environment should reflect the reality of the material in situ and should be comparable in terms of pH, ionic strength, temperature, and dilution.
- The particle size distribution of eCNF samples tends to be broad, such that the experimental conditions needed for optimal particle size measurement may differ depending on the particle size distribution of each eCNF sample, even for eCNFs produced from the same cellulose source.

OVERVIEW OF CNF PARTICLE SIZE MEASUREMENTS

As outlined earlier, there are many challenges in particle size measurement of CNF materials. Though progress has been made for iCNFs with the technical specification ISO/TS 21346:2021, there is no one widely accepted approach for eCNFs. Fortunately, approaches have been developed that have shown good utility for the particle size measurement of eCNF materials and can be used as a foundation to build upon. In 2014, Kangas et al. [9] published a "short review and evaluation" of characterization methods for mechanically manufactured CNFs that included the measurement of average particle size and particle size distribution (PSD) for five CNF samples with different degrees of fibrillation: one commercial, Daicel Celish KY-100G (Daicel Miraizu Ltd; Tokyo); two pilot scale; and two grades manufactured at VTT Technical Research Center of Finland in Espoo.

The (then) state-of-the-art measurement methods for CNF average particle size and PSD listed in the Kangas et al. [9] review were: light scattering methods (for low aspect ratio materials); transmittance by UV-vis spectroscopy or turbidity; imaging techniques using a combination of fiber analyzers, fractionation, and microscopy (i.e., SEM, TEM, or AFM). Kangas et al. studied the average particle size and PSD of the fibrillated celluloses indirectly by transmittance and directly by image analysis obtained during tube flow fractionation. SEM imaging was used to measure fibril dimensions directly. Kangas et al. concluded that a combination of both direct (SEM/TEM/AFM) and indirect (e.g., transmittance) methods is most likely required to fully quantify the CNF particle size, particularly if quantitative data is required. They suggest that appropriate sample preparation (e.g., spin-coating of substrate, sample drying) is key to particle size accuracy, but can be time consuming. Finally, microscopy methods should always be used when characterizing CNF morphology and size and could be used in combination with faster methods for quality control purposes.

More recently, a 2017 article on CNF benchmarking by Desmaisons et al. [10] suggests that a combination of the nanosized material fraction, turbidity, and transmittance at 550 nm can be used to evaluate CNF quality and provide a quality index value. However, while these methods yield data directly related to CNF dimensions, they do not measure the length and width of the CNFs, either directly or indirectly.

This section highlights the capabilities, limitations, and challenges inherent to several measurement techniques the authors consider relevant for the particle size measurement of iCNF and eCNF (**Table I**). Subsequent descriptions of these methods are classified into: direct dry imaging methods, suspension measurement methods, and other techniques. This section concludes with a brief summary of current efforts towards standardization.

Direct dry fibril imaging methods

The size range of various direct dry fibril imaging techniques is given in Table I. The lowest resolution technique is optical microscopy. While optical microscopy is not able to image the nanofiber fraction of a sample, it can be a useful method to characterize mechanically treated samples containing large fragments of the original fibers. Polarized light microscopy can also be used to enhance the resolution, although the true nanofibers still cannot be observed. Recently, a variation of optical microscopy used fluorescent staining to amplify the apparent width of iCNFs, allowing them to be optically observed [11]. The study noted that the iCNF lengths could accurately be measured, but the widths were significantly exaggerated.

To directly image the nanosize scale features of CNFs, it is necessary to use SEM, TEM, or AFM. Although electron and atomic force microscopy have been extensively used for CNM particle size measurements, standard approaches for sample preparation, imaging, and image analysis are still lacking. To address this, TEM and AFM measurement protocols have been developed for CNCs with ISO/TS 23151:2021 "Nanotechnologies—Particle size distribution for cellulose nanocrystals," and they have been used in international interlaboratory comparison studies [4,5]. These CNC size measurements are challenging due to their broad size distribution, irregular rod-shaped particles, and propensity to aggregate and agglomerate. All these challenges, among others, are even more pronounced for CNFs. Additionally, some progress has been made in automating the size analysis of less complicated materials such as CNCs and iCNFs, but there are no well-established automated techniques for eCNF samples. In addition, there is no wide-

Method	Dimension(s) Measured	Measurement Range(s)*	Analysis Time*	Standard Method Available?
AFM**	Length, height	5 nm–10 µm (length) 1 nm–1 µm (height)	30 min for single high-resolution image, additional time needed for IA and SP	ISO/TS 23151:2021 (only for CNCs)
DLS	Hydrodynamic size (related to both length and width)	1 nm–1 µm	1 h for one sample, including IA and SP	ISO 22412 (for spherical particles; not CNF specific)
Fiber analyzers (image analysis)	Length, width	~0.1–7 mm (length) ≥ 10 µm (width)	30 min for one sample, including IA and SP (also available as online versions)	ISO 16065-1 ISO 16065-2 (specific to fibers larger than CNFs)
Flow stop (rotational diffusion measurement)	Length	100 nm–10 µm	1 h, including DA and SP	None
Laser diffraction	Equivalent diameter	~10 µm–3 mm	20 min, including DA and SP	ISO 13320 (for spherical particles; not CNF specific)
Optical microscopy**	Length, width	1 µm and larger	20 min for multiple images, additional time for IA and SP	None
SEM**	Length, width	5 nm–100 µm	1 h for multiple images, additional time for IA and SP	None
SAXS, SANS (wet or dry)	Particle diameter, cross-sectional shape	1 nm–100 nm (lab variant) 1 nm–1000 nm (synchrotron)	1 h, including DA and SP	ISO 17867 (not CNF specific)
Solid state NMR**	Width	1 nm–50 nm	3 h including DA and SP	None
TEM**	Length, width	0.2 nm–5 µm	1 day including IA and SP	ISO/TS 23151:2021 (only for CNCs)
WAXS**	Crystal size	0.1 nm–1 nm	1 h including DA and SP	None

CNF = cellulose nanofibril; AFM = atomic force microscopy; DLS = dynamic light scattering; SEM = scanning electron microscopy; SAXS = small-angle X-ray scattering; SANS = small-angle neutron scattering; NMR = nuclear magnetic resonance; TEM = transmission electron microscopy; WAXS = wide-angle X-ray spectroscopy; IA = image analysis; SP = sample preparation; DA = data analysis; CNCs = cellulose nanocrystals.
* Assumed experienced operator
** Dry fibril characterization technique

I. Overview of direct methods of relevance for CNF size measurement.

spread agreement as to what measures of the statistical distribution should be reported. This is particularly problematic, given that most distributions are significantly asymmetric.

Quantifying CNF particle morphology requires high-quality images that is contingent on having a homogeneous dispersion of CNF individual particles with minimal CNF-CNF contact, minimal agglomeration, a uniform contrast across the image, and high edge contrast between CNF particles and the supporting substrate. For eCNF, obtaining such a sample configuration can be challenging, as the particles themselves are extensively branching and have a strong propensity for forming network structures (Fig. 1). Additional bundling/agglomeration of fibril branches within a single eCNF particle, as well as agglomeration between multiple eCNF particles, can occur during sample preparation when an aqueous eCNF suspension is dried on a given substrate (e.g., SEM stub, TEM grid, AFM mica substrate, etc.). Currently, there is limited capability in minimizing the fibril agglomeration and entangling during this drying process [2]. Thus, the eCNF morphology shown in dry fibril imaging methods may not be indicative of the morphology in the wet state, as the 3-D structure in suspension collapses into a 2-D shape when dried.

The high aspect ratio of eCNFs prevents both dimensions from being measured simultaneously by microscopy

and other techniques [9], not only adding to the measurement workload, but also possibly requiring separate protocols for accurate length and width determinations. Examples of imaging eCNF difficulties with SEM, FE-SEM, and TEM are shown in studies by Wang et al. [12] and Le Van et al. [13], in which only a few CNFs were present in each image, and length measurements were difficult and time consuming, requiring evaluation of tens of images per sample.

During analysis, the operator must consider the following variables: the image magnification, the number of images to capture, and the number of fibrils to image. The size distribution measured will depend on the magnification selected. At higher magnification, smaller fiber diameters can be measured; however, as the image area decreases, it becomes less representative of the sample as a whole. It can also be problematic to compare CNF size distributions when different magnifications have been used to collect the images. Therefore, it may be useful to perform analysis at multiple magnifications. Regarding the number of fibrils to image, one SEM study [14] looked at the statistics of imaging and concluded that to eliminate operator bias, all fibrils within a selected area in an image should be analyzed. Asking an operator to select 100 representative fibrils significantly increased the mean fibril diameter obtained, in comparison to asking an operator to measure all fibrils in a given area. As one example, a sample was imaged after being refined for 50,000 PFI revolutions and then homogenized for 3 passes The median fibril diameter measured from four images containing nearly 5,000 fibrils was 13 nm. Selecting 100 representative fibrils yielded median diameters ranging from 15 to 25 nm.

Scannning electron microscopy

Scanning electron microscopy is an electron microscopy technique that provides image resolution between that of optical microscopy and TEM and AFM [2]. With SEM, it is possible to image features from the millimeter size scale down to the nanometer size scale (5 nm), providing the ability to assess the different levels of hierarchical eCNF branching and measure their corresponding fibril widths [14]. However, measurement of eCNF length is challenging because of their entanglement and difficulty in detecting and differentiating the end points of fibrils. Typically, eCNF samples for SEM must be coated with a thin conductive layer, which subsequently limits the smallest feature size that can be imaged. Protocols for the SEM sample preparation for imaging eCNF are given in Foster [2] and Ang [14]. Additionally, contrast SEM imaging has been used to generate high contrast images between the highly conductive imageable substrate and the uncoated non-conductive eCNF, facilitating analysis of nano-sized fibril structures as narrow as 20 nm. Protocols for contrast SEM imaging sample preparation are given in Mattos et al. [15].

Transmission electron microscopy

Transmission electron microscopy is a high-resolution electron microscopy technique based on the transmission of electrons through the sample, with resolution typically down to 1 nm. Cellulose has low contrast in TEM, as it is a low electron density material, and thus often must be doped with a contrast agent to be clearly visualized. Protocols for doping CNF for TEM have been established [2,6]. Frozen suspensions can also be imaged using the Cryo-TEM technique. A thin sheet of the suspension is amorphous frozen, most often by plunge freezing, providing very low imaging response from the water, and this gives greater contrast to the objects in suspension. This is a powerful technique that allows CNFs to be imaged in the wet state. The technique is very appropriate for iCNFs, as the higher resolution of the TEM is needed to measure their small widths, and it is possible to prepare imaging layers (coatings/sheets) of small enough thickness. The thickness of the imaging layer is often required to be at most some tens of nanometers in order to provide high contrast images. Many eCNF material features are larger than this size limit. Additionally, the high resolution provided by TEM is often not needed for eCNFs. As the sample preparation is much more cumbersome than for SEM and TEMs are more expensive and more difficult to operate, SEM is often more suitable for eCNF characterization. However, there are a few studies in which eCNFs have been examined by TEM [12]. For example, Fall et al. imaged one eCNF sample (enzymatic pre-treated CNF, abbreviated NFC120 in the article) by Cryo-TEM and compared it to two iCNF samples, and the larger particle width distribution of the eCNF was easily detected [16].

Atomic force microscopy

Atomic force microscopy is often used to measure CNC and iCNF dimensions, in particular particle heights, relying on the high sensitivity of the technique in the z-direction. This approach creates topographical maps, scanning line-by-line to build up an image, which can be time-consuming depending on the scan size and image resolution. The AFM scan areas are often 10 µm × 10 µm or smaller, which means that for eCNFs and other long, entangled particles, their end-to-end lengths are difficult to capture. In addition to requiring sharp probes and appropriate settings for imaging, the quality of AFM images depends on the uniform deposition of dilute suspensions onto flat substrates, often mica or silica wafers. Commonly, the substrates are first modified with cationic polymers (e.g., poly allylamine hydrochloride [PAH] or polyethyleneimine [PEI]) to promote the deposition and adhesion of the cellulose nanomaterials, which are deposited in a subsequent stage. Spin-coating, dip-coating, and drop casting are used to deposit the materials, usually with a water rinse between the additions of cationic polymer and cellulose nanomaterial. Protocols for the dip-coating and AFM sample preparation are given in Mattos et al. [15].

Variation in CNF heights and lengths, coupled with the nanoscale focus of AFM, significantly hinders accurate imaging of representative samples. The z-scale is typically large (+/- 150 nm) in order to capture both the large fibril bundles and the finer individualized nanofibrils. As with other techniques, eCNF lengths are not easily captured in the image; however, the high nanoscale resolution facilitates visualization of individualized nanofibrils and the quantification of their heights, corresponding to the fibril width. Overall, difficulties in applying AFM imaging to quantify eCNF size relate to large variations in z-scale within the scan area that prevent accurate topographical tracking; limitations in capturing eCNF lengths due to entanglement; fibrils extending beyond the confines of the scan area; long scan times; multi-step sample preparation; and the requirement for a skilled operator to run the instrument and process the images.

Suspension measurement methods

Automated optical fiber analyzers

Automated optical fiber analyzers have gained interest for analyzing CNF samples in suspension. The analyzers typically use crossed polaroids to directly image fibers flowing through a capillary. Modern fiber analyzers will report fiber length, width, and characteristics such as fibrillation. As an example, a Valmet FS5 fiber image analyzer (Valmet; Espoo, Finland) has been used to obtain images of eCNFs produced by grinding with minerals [17]. Length-average particle lengths were determined and used to assess the effect of increasing length on the tensile strength of CNF nanopaper. The measurement is typically rapid, as the analyzer is designed for high throughput applications in the paper industry. However, it is important to note that while the analyzer camera captures thousands of images, it is only sensitive enough to measure CNF particles with lengths of 100 µm and greater, limiting the application of this technique to determine the larger fragments remaining in a sample from mechanical treatment.

Dynamic flow method

A dynamic flow method analogous to a fiber image analyzer has also been investigated for iCNFs [18]. With this method, suspensions of iCNFs flow across a microfluidic chip with 100–300 µm wide channels, and images are captured by an electron-multiplying charge coupled device camera. Although this method eliminates sample preparation artifacts and could potentially be used online to measure a large number of particles, image analysis is required, and resolution and automation issues must still be overcome.

Field-flow-fractionation

Field-flow-fractionation (FFF) has been used to separate heterogeneous fiber mixtures into fractions. This has been combined with multi-angle light scattering to determine particle lengths of the different fractions. It is worth noting that ISO/TS 21362:2018 "Nanotechnologies—Analysis of nano-objects using asymmetrical-flow and centrifugal field-flow fractionation" provides general guidelines and FFF procedures for nano-objects. A 2018 study by Isogai et al. [19] examined iCNFs of different lengths; however, iCNFs with lengths > 300 nm could not be separated properly by the FFF system.

Laser diffraction

Laser diffraction was used by Berto and Arantes [20] to study pulp defibrillation during ultra-refining in a Super-MassColloider. Changes in the size (length, hydrodynamic diameter) distribution profile were expected throughout the defibrillation process, but the size distribution pattern remained practically constant. Although the cellulose fibers underwent delamination in the longitudinal direction, reducing the fiber diameter, the fiber length was preserved, leading to essentially no change in the measured hydrodynamic diameter. Only at a higher degree of defibrillation (at 30 kWh/kg) could the effect on particle size distribution be observed with laser diffraction. To increase their understanding of the morphological changes occurring, they complemented the laser diffraction measurements with optical microscopy images and AFM images, and with other, non-size-related measurements.

Dynamic light scattering

Similar to laser diffraction, dynamic light scattering (DLS) cannot be used to measure the exact CNF dimensions (length, width), since the standard data analysis method assumes spherical particles. Even though there are models for other shapes, they are not regularly included in DLS software. In addition, there is no standard "stiff or flexible rod" shape that fits CNFs, especially not eCNFs, as they are branched and entangled and contain kinks. In contrast, DLS can work for CNCs, as the stiff rod assumption is reasonable. Since a small amount of very large particles present in the measurement sample can obscure the contribution of smaller particles, filtration or centrifugation of samples prior to measurement is often required [21]. In other cases, many different fractions can be obtained and analyzed. The measured hydrodynamic radius can be affected by the surface charge density and ionic strength of the liquid. In addition, it is difficult to fully individualize CNFs, particularly eCNFs, and the suspensions will mainly consist of CNF aggregates. Therefore, the translational diffusion (raw data of DLS) will be more related to the aggregate size than the size of individual CNFs. However, DLS is suitable for following changes in CNF size with varying treatment/chemical conditions [20,22]. Thus, even if the dimensions cannot be quantified, the size change can be monitored.

Shear viscosity

Shear viscosity has also been used to calculate nanofibril length for iCNF samples with different average lengths by

applying an equation for the dilute region flow behavior of rod-like polymer molecules to the shear viscosities of the dilute dispersions. A linear relationship of the viscosity-average length to the length-weighted average iCNF length measured by microscopy was found [6]. For eCNFs, the relationship between shear viscosity and particle morphology is less clear. Viscosity generally increases with increased fibrillation degree due to decreased particle size; however, concurrent changes in several other variables, including particle aspect ratio, particle branching, size distribution, and formation of network structures make it challenging to directly relate a given rheology profile to a meaningful particle size [9]. Despite this, rheology can be used to measure mechanical properties, such as gel strength, to estimate floc size, particle concentration, etc.

Turbidity

Turbidity can be used as a measure of the relative average particle size during different nanofibrillation processes, for example. It is often measured together with other properties, such as optical microscopy and nano-sized fraction obtained by centrifugation. The advantage with turbidity and centrifugation over many other methods is that they can give reliable data throughout the whole manufacturing process [21]. Turbidity measurements have been used to estimate CNC and CNF diameters [23], although the maximum estimated size of the samples tested was 10 nm. The results showed a reasonable relationship between the diameter estimated from turbidity and the height measured by AFM. The LUMisizer (LUM Gmbh; Berlin), an instrument that combines optical transmittance with centrifugation at varying speeds, can be similarly applied to address colloidal stability and nanoyield [24].

Gel point sedimentation

Gel point sedimentation is used for mechanical CNF aspect ratio measurement. The measurement relies on determining the lowest solids content at which a nanofiber suspension forms a connected network. One of the most widely used methods to do this is sedimentation [25]. In this technique, a series of dilute suspensions with different solids contents are prepared and allowed to settle. The data is then plotted as the initial concentration vs. the ratio of the final settled height to the initial height. The data typically does not show a straight-line relationship between concentration and settled height, due to the compaction of the sediment with increasing concentration, and extrapolation to the origin is required. The gel point of four batches of eCNFs estimated by sedimentation showed good agreement with that measured from the yield stress in vane rheometry [25]. The gel point generally decreases with refining and homogenization or grinding [26,27]. A decrease in gel point means that aspect ratio is increasing and therefore that the diameter is decreasing faster than the length. Aspect ratio can be calculated from the crowding number or from the effective medium theory [25].

Water retention value

Water retention value (WRV) can be used for indirectly assessing the degree of fibrillation of mechanical CNFs. The WRV has been used widely in the pulp and paper industry to characterize the extent of fibrillation of fibrous materials with lengths in millimeters and radial dimensions of tens of micrometers. This method was successfully adapted by Gu et al. [28] for the indirect assessment of the degree of fibrillization of mechanical CNFs, the validation of which was based on comparisons with SEM, TEM, enzymatic absorption, and Canadian Standard Freeness. The practical significance of the WRV method is that it is nondestructive, simple to implement, uses inexpensive equipment, and typically takes less than 30 min per test.

Other techniques

Small angel scattering

Small angle scattering (SAS) is a non-destructive indirect method providing nanoscale structure information from 1 nm to 100 nm over a complete volume [29-31]. The SAS is capable of measuring CNFs and CNCs in any form of powder, foam [32], films [33], gels [34], and suspension [35]. In SAS, the scattered X-rays from small-angle X-ray scattering (SAXS) or neutrons from small-angle neutron scattering (SANS) of the sample contain information on nanostructure geometry, distribution, interparticle interactions, assembly, correlation length, volume fraction, number density, and fractal formation [36,37]. The obtained SAS curve is a plot of scattering intensity as a function of scattering vector q. The lower q values in the curve reveal information on the nanometer scale dimensions, and the higher q values give information of the atomic scale dimensions and interactions.

The SAS method has been used to assess the morphology of CNFs extracted from different sources by different methods. Mao et al. used SAXS to compare the morphology of CNCs and CNFs extracted from wood by sulfuric acid treatment and TEMPO oxidation, respectively [38]. The CNCs had a width of 20 nm and length from 100 to 150 nm, while the TEMPO-oxidized CNF was 8-nm wide and 2-nm thick with broad length distribution. Likewise, Geng et al. modeled SAS curves with the ribbon model and found that TEMPO-oxidized CNFs from jute fibers were 7-nm wide and 1.5-nm thick [39]. An analysis of SAS curves for enzymatic hydrolyzed bacterial cellulose showed similar ribbon shape and decrease in intensity with hydrolysis time. The well fitted unified model shows two sizes of CNF in terms of radius of gyration: first, the elemental CNF of size 3–4 nm, and second, the large size CNF of 26–30 nm [40]. Thus, SAS techniques successfully provide information on the shape and diameter of CNFs in as-prepared samples. However, to obtain meaningful data, significant expertise in fitting the SAS curves by choosing appropriate shape models is required.

Surface charge

The surface charge of anionic CNFs is usually measured by polyelectrolyte adsorption using a streaming potential titration [41]. The most common polyelectrolyte used for this purpose is poly(diallyldimethylammonium chloride) (PDADMAC), having a known charge per unit volume (μeq/mL). Unlike the total charge, in which the CNF suspension is titrated by sodium hydroxide (NaOH), a molecule small enough to penetrate fibril bundles, the polyelectrolyte titration only probes surface accessible charge groups. The more fibrillated the CNF, the more the total charge and surface charge values approach one another, and vice versa [16]. In this way, the comparison of total charge and surface charge can be a useful technique to understand the degree of fibrillation of CNFs.

Towards standardization

The wide variety of techniques available for CNF particle size measurement, each with its own strengths and limitations, indicates that there will be no "one size fits all" standard method for CNF particle size measurement. The CNFs also present additional challenges in dispersing to obtain individual particles, and their inhomogeneity may necessitate fractionation prior to characterization [9,19,42]. Thus, sample preparation must also be specified in any standard method developed. The ISO technical specification for iCNF, ISO/TS 21346:2021, gives sample preparation, measurement, and data analysis procedures for size measurement by TEM and AFM. However, there is no corresponding ISO document for eCNF materials.

Direct particle size measurement techniques such as TEM and AFM appear to be the "gold standard" for length and width determination, as microscopy data is frequently compared to data obtained by other methods [6,19]. However, microscopy methods possess their own considerable inherent limitations and challenges that must be addressed: they measure small, potentially non-representative samples, require significant expertise, and are laborious, expensive, and more or less subjective.

Given this state of affairs, it is then necessary to establish priorities as to which methods should be developed as standards for eCNF particle size measurement. Some criteria to be considered for standardization could include: commercial systems currently available, methods that are widely used, methods that do not require extremely specialized skills, and/or methods that give easily interpreted data. The consultation process to establish these priorities is discussed in the following sections.

SURVEY OVERVIEW AND RESULTS

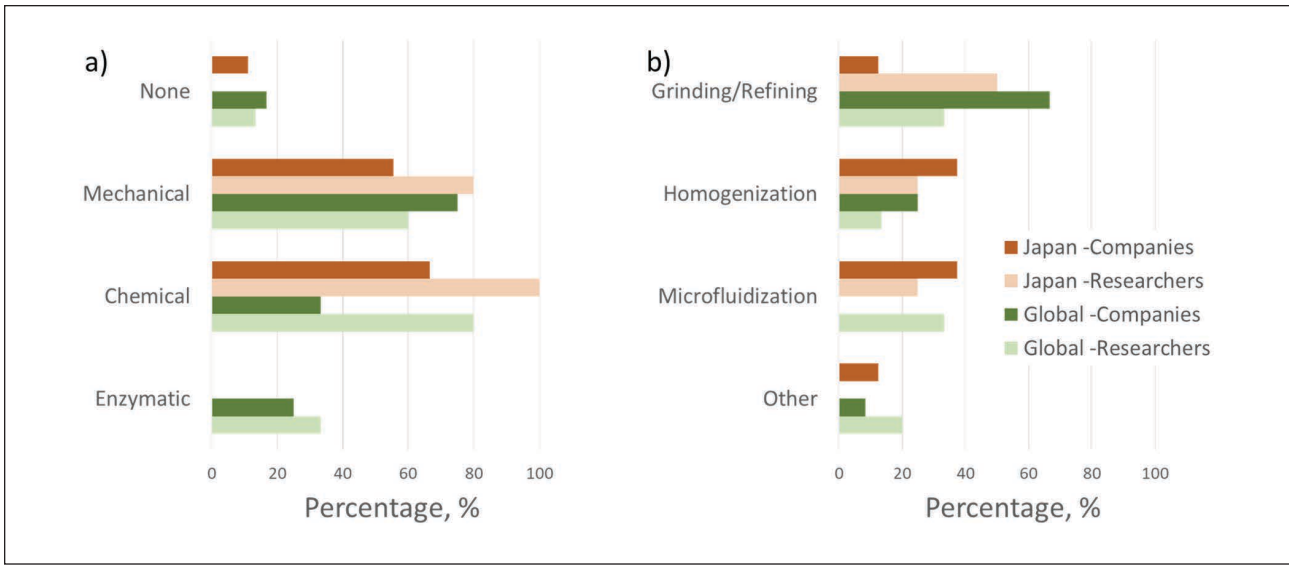
Survey structure

In 2020, an online survey was distributed to international CNF producers, end-users, and researchers. The survey was developed, carried out, evaluated, and reported by ISO/TC 6/TG 1 members representing industry, academia, and federal research laboratories in six countries. Survey questions were centered around three themes: respondent details, CNF processing information, and CNF particle size measurement needs. Respondent details were used to better understand responses, such as: were they from research institutions or companies; were they a CNF producer, end-user, or supplier; and in which industry segment(s) did they work. The CNF processing information provided context as to the CNF type. Participants were asked about their pretreatment step (none, mechanical, mechanical and chemical, chemical, enzymatic); final fibrillation step (grinding/refining, microfluidization, homogenization, other); and characteristics of the final CNF product, in particular the relative liquid content (fluid suspension, gel, paste, dry) and the relative degree of dispersion (individualized fibrils, entangled fibrils).

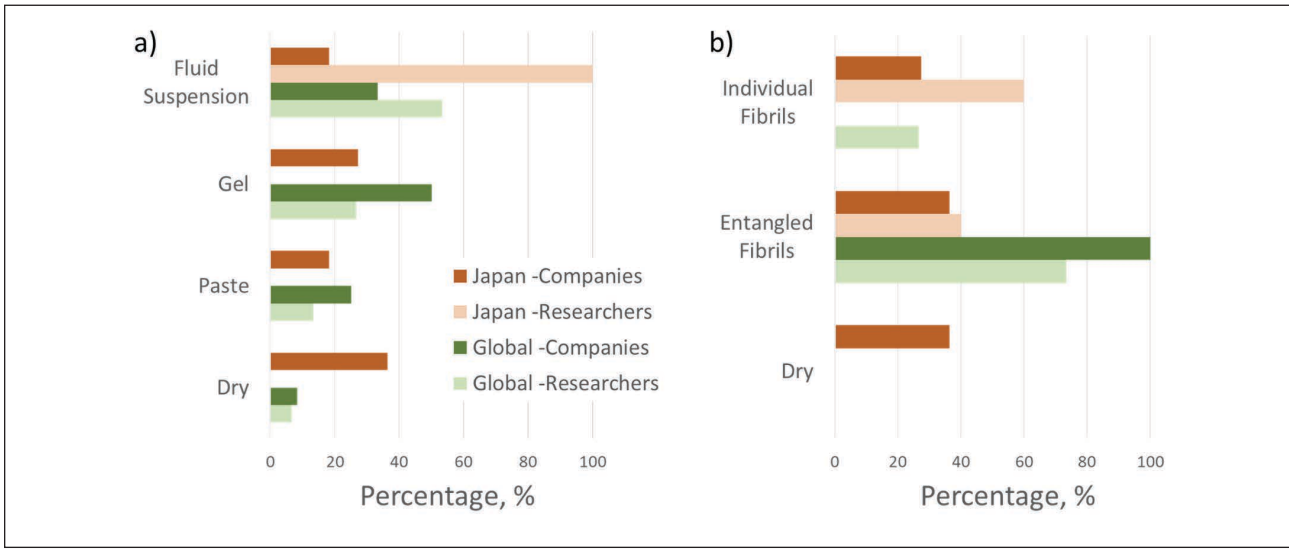
Finally, participants were asked if CNF particle size measurements were needed for safety/regulatory compliance, product specification, grade definition for patents or benchmarking, production control, or any combination of these purposes. For each need, participants were asked which specific particle size measurements were needed (length, width, aspect ratio, hydrodynamic diameter, and other). The participants were also asked which were their preferred methods for reporting particle size and size distribution (average and standard deviation, continuous distribution, Gaussian distribution, percentiles D(10), D(50), D(90)). The participants were requested to consider if the CNF particle size measurements needed to be carried out in the wet state (i.e., in suspension), and whether the effects of salts and additives needed to be accounted for. The participants were also asked if they were currently measuring CNF particle size, and by which techniques.

Survey distribution

An online platform was used for this survey, to which participants gained access via a weblink. The online survey was distributed to a wide scope of CNF producers, end-users, and researchers working with CNF materials. The survey was anonymous, thus relating results back to the responding institution was not possible. Due to the increased focus of iCNF in Japan and the increased focus of eCNF in the rest of the world, the survey participants were separated into two groups. The "Japan" group consisted of participants employed at institutions located in Japan, while the "Global" group consisted of participants from the rest of the world. To facilitate participation from the Japan group, the online survey notification was coordinated by TAPPI Japan and Nanocellulose Japan (NCJ). Their members were notified via email and provided a website that contained a description of the survey, a copy translated into Japanese, and a link to the online survey. Notification to the Global group was coordinated by ISO and the TAPPI Nanotechnology Division via an email distribution list, with open participation.



3. For all survey respondents: a) pretreatment step, and b) final fibrillation step used in their CNF production method.



4. For all survey respondents: a) relative liquid content, and b) relative degree of dispersion in their final CNF product.

Survey results

There were a total of 48 respondents, 17 from the Japan group and 31 from the Global group. For the Japanese respondents, there were 5 from research and 12 from companies, of which 5 were from the chemicals industry, 3 from the forest products industry, 3 from equipment manufacturers, and 1 from services. For the Global respondents, 17 were from research and 14 from companies, of which 10 were from the forest products industry, 2 from services, 1 from consumer goods, and 1 from the chemicals industry.

Participant responses on CNF processing information confirmed that CNF production in the Japan group was different from that of the Global group—and there were also differences between companies and researchers. Pretreatments were overwhelmingly used by Japanese and Global companies and researchers, with chemical and mechanical pretreatments dominating (**Fig. 3a**). In general, CNF production in the Japanese companies consisted of chemical and/or mechanical pretreatments with a final fibrillation step of homogenization or microfluidization, while production in the Global companies consisted of mechanical pretreatment with a final fibrillation step of grinding/refining; the ramification of these differences in CNF production approaches is that the final CNF morphologies will be expected to be different for the two groups. (Fig. 3b). In contrast, Japanese and Global researchers used a larger variety of methods as their final fibrillation step (homogenization, microfluidization, grinding/refining, or other).

Participant responses regarding the characteristics of the final CNF product varied widely. With respect to the relative liquid content of the final CNF product (fluid suspension, gel, paste, dry), the Japanese and Global companies, as well as

	Companies		Researchers	
	Japan	Global	Japan	Global
Production control	58% W (90%) L (65%) A (55%)	43% L (90%) W (75%) A (70%)	80% L (80%) W (80%) A (40%)	71% L (90%) W (75%) A (65%)
Safety/Regulatory requirements	33% W (89%) L (67%) A (67%)	71% W (100%) L (90%) A (30%)	20% L (100%) W (67%) A (67%)	47% L (92%) W (92%) A (58%)
Product specification	58% W (92%) L (75%) A (58%)	57% L (75%) A (67%) W (58%)	100% W (80%) L (60%) A (60%)	76% L (94%) W (88%) A (69%)
Grade specification	50% L (78%) W (78%) A (67%)	71% L (85%) W (69%) A (62%)	20% W (80%) L (60%) A (40%)	53% L (92%) W (85%) A (58%)
Benchmarking	50% W (80%) A (80%) L (70%)	20% L (100%) W (92%) A (67%)	36% W (100%) L (75%) A (75%)	59% L (93%) W (87%) A (60%)

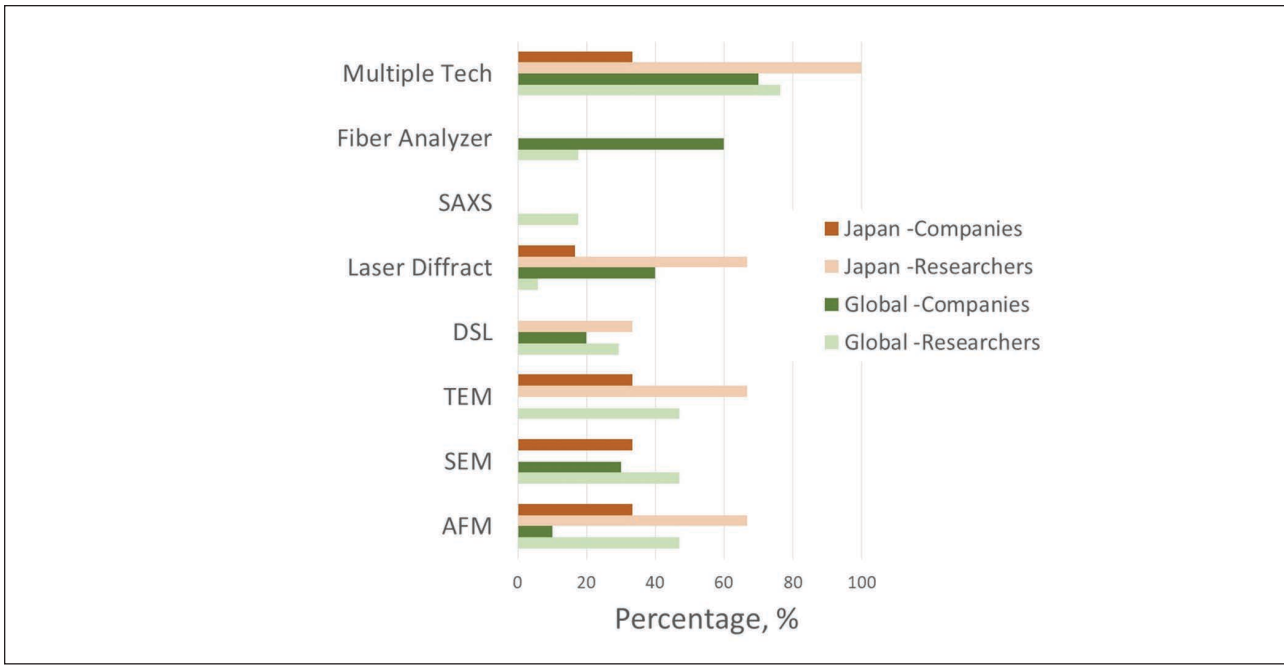
CNF = cellulose nanofibril; W = width; L = length; A = aspect ratio.

II. Summary of survey results for CNF particle size measurement standardization needs and the particle dimension needed. For each category, the following information is listed: 1) the top number is the percent of participants believing that a particle size standard was needed for this given area of interest, and 2) the bottom numbers show the percent of participants that believe the given particle dimension was important to measure, listed in decreasing order of importance.

Global researchers, produced materials having relative liquid contents, ranging from fluid suspensions to dry powders. In contrast, Japanese researchers focused only on fluid suspensions (**Fig. 4a**). It was interesting to note that the predominant product reported by companies was a dry CNF product by Japan (35%) and a gel CNF product by Global (50%). As to the relative degree of dispersion of the final product, the Japanese companies and researchers produced both individual (iCNF) and entangled fibrils (eCNF), at similar levels (Fig. 4b). In contrast, Global companies and researchers used primarily entangled fibrils, with Global companies responding that they only produce eCNFs. In general, iCNFs are more common in Japan than in the rest of the world, indicating that CNF particle size measurement method needs may be different in Japan compared to the rest of the world.

Participant responses indicated that particle size measurements standards were needed for safety/regulatory, product specification, grade definition for patents, benchmarking, production control, and any combination of these, but the priority levels were different between the Japan and Global groups, as listed in **Table II**. Japanese companies indicated their greatest needs were for product specification, production control, grade definition for patents, and benchmarking, while for Japanese researchers, the predominant needs were product specification and production control. In contrast, Global companies listed their greatest needs as safety/regulatory, grade definition of patents, and product specification, while for Global researchers, the greatest needs were product specification and production control, as in Japan. For each standardization "need," the top three priority particle size measurements, with the percent voting in parentheses, are summarized in Table II. In general, length and width were highest priority, with aspect ratio having a somewhat lower priority, and hydrodynamic radius having the lowest priority; thus, hydrodynamic radius was not included in the data in Table II.

Preferences for how particle size measurements should be reported indicate that participants from all groups overwhelmingly wanted the size distribution to be reported for length and width measurements (> 70%), while Global companies also wanted the size distribution for hydrodynamic radius (~65%). The preferred type of distribution informa-



5. Techniques used by respondents to measure CNF particle size (SAXS = small-angle X-ray scattering; DLS = dynamic light scattering; TEM = transmission electron microscopy; SEM = scanning electron microscopy; AFM = atomic force microscopy).

tion to be reported was somewhat mixed. The average value and standard deviation approach was favored by Global researchers (85%), Japanese companies (70%), and Japanese researchers (60%), while percentile approaches were favored by Global companies (65%) and Japanese companies (65%).

Current participant particle size measurement activity indicates that the majority of participants measure CNF particle size, with Japanese and Global companies around 70%, Global researchers at 100%, and Japanese researchers at 60%. Participants reported that they typically use multiple techniques to measure CNF particle size (**Fig. 5**). Japanese companies predominantly use microscopy methods including AFM (30%), TEM (30%), and SEM (30%), while Global companies primarily use fiber analyzers (60%), laser diffraction techniques (40%), and SEM (30%). This notable difference in the measurement methods between Japanese and Global companies is indicative of differences in the predominant CNF materials produced and used (iCNF and eCNF, respectively). Japanese researchers use AFM (65%), TEM (65%), and laser diffraction techniques (65%) to measure CNFs, while Global researchers primarily use microscopy methods, including AFM (45%), TEM (45%), and SEM (45%). Differences between methods used by Global companies and researchers likely stem from the differences in cost, time, and skill level required by the various techniques.

Participants from all groups overwhelmingly agreed that particle size measurements were needed for fully dispersed CNF suspensions as follows: Japanese companies (82%), Japanese researchers (100%), Global companies (90%), and Global researchers (70%). Responses regarding the need to account for salts (e.g., hydrochloric acid [HCl] and sodium chloride [NaCl]) and additives in CNFs when carrying out particle size measurements were mixed as follows: Japanese companies (30%), Japanese researchers (40%), Global companies (42%), Global researchers (75%).

Discussion of survey results

The stated purpose of the standardization work within ISO is to "help companies to access new markets, […] facilitate free and fair global trade, and ensure that products […] are safe, reliable and of good quality." Therefore, our discussion of the survey results focuses on the needs of the companies, rather than those of the researchers.

The motivation for CNF particle size measurement standardization differs between Japanese companies and those in the rest of the world ("Global companies"). Global companies were more interested in standards for safety/regulatory and patenting purposes than Japanese companies, probably due to differences in their CNF products. For Global companies, there may be a need to classify the materials they produce as nanomaterials or non-nanomaterials, while the iCNFs that are commonly produced in Japan are more obviously classed as nanomaterials. While Japan is more interested in production control than the other countries, all countries, including Japan, are interested in product specification.

Overall, companies around the world, including Japan, agree that particle size measurement standards will eventually be needed for all these purposes. They also agree that CNF width and length are the top priority measurands, which is in agreement with researchers (Table II). Accurate measurement of these basic CNF dimensions is crucial to

identifying nanoscale material, including entangled nanofibers in CNF/MFC. Separating entangled nanofibers could be a future and more challenging step when developing standard methods. On the other hand, for process control, a rapid length and width testing method is required to define the degree of fibrillation. Aspect ratio is medium priority (except for being indicated as high priority for benchmarking along with width and length), while hydrodynamic radius is low priority, possibly as it is difficult to measure and interpret, particularly with opaque dispersions of polydisperse CNFs.

Interestingly, CNF researchers appear to be product- and market-focused, or possibly at least focused on the needs they think the industry has. What is good for the industry is also considered to be good for them. An example is that researchers, even to a greater degree than the industry, think that product specification is an important area for standards development. This is especially the case in Japan (Table II). In recent years, research has been increasingly targeted to the future needs of industry; collaboration with, and financing from, industry has also become more common.

WORKSHOP OVERVIEW AND RESULTS

Overview

To obtain broader perspectives on standardization needs for CNF particle size measurement directly from the world community, a two-day virtual workshop, "Workshop on cellulose nanofibril particle size measurement: Setting priorities in standard development," was co-hosted by ISO/TC 6/TG 1 Cellulosic Nanomaterials and TAPPI's Nanotechnology Division on September 14-15, 2021. There was a strong attendance of 65 registrants each day, representing industry, academia, and federal research laboratories from 10 countries. In total, 30 institutions, of which 15 were from industry, participated in the workshop. All of these factors helped ensure active dialogue and broad global perspectives on the standardization needs for CNF particle size measurement.

Day 1 was informative in nature, focusing on i) the findings of the 2020 survey (see Survey Overview and Results section), ii) size measurement techniques, and iii) eCNF measurement examples. To provide a framework for CNF morphology and size characterization, experts in the field gave introductions on the capability and effectiveness of various techniques used to measure CNF particle size, the measurement challenges faced by iCNFs and eCNFs, and their corresponding feasibility in industry vs. research institutions. Separate breakout rooms led by experts were created to allow in-depth discussions on the state-of-the-art of eCNF particle size measurement, which eCNF features to measure, measurement techniques/approaches, and the identification of priorities in standards development needs.

Day 2 was centered on four interactive discussions guided by the SWOT methodology, in which the parameters Strengths, Weaknesses, Opportunities, and Threats provide a structured framework to the discussion of a given topic. Based on the findings of the 2020 survey, the following SWOT discussion topics were covered in the workshop: CNF diameter measurement needs, CNF branching measurement needs, CNF size measurement for product specification needs, and CNF size measurement for safety/regulatory needs. The CNF branching and diameter SWOTs were more fundamental in nature, while the product specification and safety/regulatory SWOTs were more focused on applied development. The outcomes for each discussion group were itemized lists covering Strengths, Weaknesses,

Strengths	Weaknesses
• Many different types of techniques that can be potentially used to measure diameter • Diameter of coarser fractions is easily measured using standard techniques • Size measurement standards for other nanomaterials may be applicable	• No acceptable definition of "diameter" • Material is highly complex • Techniques rely on skilled operators and require long analysis times • Rapid techniques are indirect, and it is unclear how the results are correlated to the diameter
Opportunities	**Threats**
• Development of automated or semi-automated analysis • Identify which features within eCNF should be measured for size • Significant interest and method development activities within industry; should be possible to find common ground	• Difficult to measure and identify relevant size scales (arbitrariness, bias) • No consensus on diameter definition • Other parameters may turn out to be more important than diameter; for example, specific surface area

6. Key points from CNF diameter SWOT discussion.

Strengths	Weaknesses
• Many different techniques can potentially be used to measure CNF branching	• Complex and difficult problem to solve • Unknown what properties in products are affected by branching
Opportunities	**Threats**
• Area is wide open, with a large area of interest in the community • Even partially addressing this issue would be well regarded • Developing a standard set of samples that can be measured by different techniques; these need to cover many different length scales (tiny to large branches) • Developing indirect techniques to complete these types of measurements in a timely manner • Potential to bring across techniques from other fields, e.g., fractal characterization	• Important hidden variables determine eCNF quality; without measurement capabilities it is impossible to fully specify quality • No consensus on how to systematically define branching

7. Key points from CNF branching SWOT discussion.

Opportunities, and Threats, as well as a list of future activities/tasks related to the development of guidelines, updating of existing standards, or development of new standards. Detailed descriptions and outcomes for each SWOT are given in the following subsections.

eCNF diameter measurement needs

The CNF diameter SWOT discussion was led by Dr. Andreas Fall (RISE Research Institutes of Sweden; Gothenberg), Dr. Gustav Nyström (Empa; Dübendorf, Switzerland), and Dr. Tiffany Abitbol (also of RISE). The key outcomes are listed in **Fig. 6**. Two main tracks for diameter measurement were discussed: rapid and most often indirect methods for process control vs. slower but accurate/quantifiable methods for product specification and/or regulatory demands. The former included laser diffraction and turbidity measurements and image analysis using fiber analyzers. The latter are typically SEM methods, but AFM was also briefly discussed. Many aspects of sample preparation, including the use of fractionation, were also part of the discussion. It became clear during the discussion that there is significant development currently underway within various companies. Collaborations within ISO could help to find the common ground among the needs of the various sectors and the capabilities of each technique.

Regulatory agencies, as well as new customers, generally do not understand the complexity of the various CNF materials. The ISO could help to educate the community, particularly regulatory agencies, about key aspects of CNF materials and the applicable measurement methods.

There was a lot of discussion of what exactly was meant by "diameter," and it was clear that this parameter must be further defined. We tried to focus the discussion on the nanosized range of diameter measurement. Measurement guidelines for diameter determination were seen as a highly interesting and desirable future deliverable from ISO. It was also requested that a list of laboratories capable of performing the suggested characterizations be compiled. Wet and dry measurement methods were also discussed, given that sample preparation for dry methods can alter the CNF diameters being measured due to aggregation and agglomeration of the cellulose fibrils. X-ray was discussed as a possible method for quantifying CNF diameter in the wet state. Finally, correlating the results obtained via various indirect measurement methods with CNF diameters measured by microscopy was of high interest. How to define these correlations could be included in the guidelines, as these faster indirect methods are crucial for process control.

eCNF branching measurement needs

The branching SWOT discussion was led by Professor Warren Batchelor (BioPRIA, Monash University; Clayton, VIC, Australia), Dr. Andreas Fall (RISE), and Christine Browne (BioPRIA). The key outcomes are listed in **Fig. 7**. There was widespread agreement that the CNF "branching" feature is important to measure and that current measurement techniques are lacking. One of the difficulties in quantifying branching is that while it is extremely difficult to preserve the branched structure of the nanofibers that is present in aqueous suspension when they are dried, the main direct

Strengths	Weaknesses
• Large interest and need for product specifications • "Bulk methods" are representative of a large part of the sample • Indirect methods (e.g., viscosity, transparency, turbidity, nanofraction assessment, accelerated sedimentation) are straightforward and accessible	• Presence of other materials (e.g., lignin, hemicellulose, pigments) may require either modification or different methods • TEM/SEM/AFM are labor- and time- consuming and not preferred by industry • Difficulty in relating dry and wet fibril dimensions
Opportunities	**Threats**
• Microscopy as a complement to qualitatively classify eCNFs according to morphology • Automatic or semi-automatic image analysis techniques for length and width can increase the feasibility of imaging methods for the industry • Dry CNFs can possibly use existing standard methods for various powders, or develop these further	• Indirect methods fail to capture some metrics that are needed/wanted, e.g., number distribution of particle size • If wet material is characterized by industry and then sold dry and redispersed elsewhere, the initial material will likely be changed

8. Key points from product specification SWOT discussion.

measurement techniques (SEM, TEM, and AFM) require dried samples. The eCNFs tend to coalesce and entangle with each other as they are dried. In addition, even if there was no coalescence, the three-dimensional (3D) structure will collapse to form a two-dimensional (2D) projection during drying. While we cannot prevent the collapse from a 3D to 2D structure, it will be critical to develop techniques that can prevent coalescence in drying. It will also be critical to develop indirect measurement methods that can be applied in suspension.

It is likely that characterization of eCNF branching will require combining multiple measurement techniques to fully evaluate branching. Therefore, it is critical that eCNF preparation methods be developed to systematically vary branching, so as to be able to prepare sets of eCNF samples that can be analyzed with different techniques in different laboratories to allow comparison and correlation among techniques.

Product specification

The product specification SWOT discussion was led by Dr. Cecilia Land Hensdal (Stora Enso Pulp & Paper Asia AB; Karlstad, Sweden), Julio Costa (WebTech Pulp & Paper Technologies; Piracicaba, SP, Brazil) and Dr. Tiffany Abitbol (RISE). The key outcomes are listed in **Fig. 8**. This SWOT topic was more relevant to applied development, and the discussion began by identifying various measurement needs connected with product specifications and what types of characterization methods industry would be willing to adopt. For product specification, bulk methods were preferred over microscopy methods (e.g., optical microscopy, SEM, AFM, and TEM). Imaging methods only examine a limited part of the sample, and they are time-consuming and difficult to perform. Regardless, optical microscopy could be used as a complementary method to establish a qualitative classification of eCNF materials according to morphology.

Indirect methods were a preferable alternative to direct size measurement in some cases, especially if calibration or correlation with the size obtained by direct measurement is performed. However, it was noted that impurities or other materials present (e.g., hemicellulose or lignin) can interfere with some measurement methods, especially optical measurements that require the refractive index of the material for calculations. Different standard methods may thus be needed for eCNF qualities containing residual or added lignin.

When reporting a product specification result, it is important to specify if the measurement was carried out on never-dried material or dried and redispersed material. If never-dried material is characterized by industry and then sold dry and redispersed elsewhere, the properties will likely be different. If measurements on dry powder CNFs are needed, perhaps existing standard methods for different powders can be used or developed further.

Safety and regulatory requirement

The safety/regulatory requirement SWOT discussion group leadership was Dr. Robert Moon (USDA-Forest Service; Atlanta), Professor Heli Kangas (VTT Technical Research Center of Finland; Espoo), and James Ede (Vireo Advisors; Boston). The key outcomes are listed in **Fig. 9**. The key threats and weaknesses were discussed first and centered around the ambiguity of regulatory requirements and lack of clarity of what to measure from the various safety and

Strengths	Weaknesses
• Already have some success with eCNF regulatory applications • AFM, SEM, and TEM have width measurement capability and statistical relevance to address regulatory needs (~1 nm) • Fractionation techniques have capability to isolate nanoscale fraction to address regulatory needs (fraction of nano-objects)	• Cannot isolate single eCNF particles • Unknown what to measure to satisfy regulatory needs • Unclear what is the relevant eCNF dispersion state required for measurement
Opportunities	**Threats**
• Terminology — avoid "nano" altogether? • Redefine CNF • Build from successful effort in CNF regulation • Benchmark with "traditional" forms of cellulose currently in use • Consult with regulators throughout process • Regulatory agencies can be educated on the complexity of eCNFs, their branching, and the relevancy of their hierarchical structure	• Regulatory requirements: - Vary in information requested - Depend on country and agency - Constantly change • Regulator's simplistic view of eCNF structure results in requests to quantify features that cannot currently be measured • Health and safety issues due to nanoparticle entrainment (branched CNFs grab onto other particles)

9. Key points from safety and regulatory SWOT discussion.

regulatory agencies. Characterization requirements are use-case dependent (e.g., food, food contact, chemical registration, etc.), and they may require characterization in different states (e.g., powder, gel) and matrices (e.g., food). Thus, the requirements differ depending on the regulatory bodies, the agency, and the country. For example, the European Unoin requires number concentrations of different particle sizes, whereas the United States requires weight/mass concentrations of different particle sizes. Additionally, the requirements are constantly changing. All these factors make eCNF particle size measurement requirements into a moving target, and as such, a robust approach will be needed to have the flexibility to address the various needs as they evolve.

Also noted as a threat is the fact that regulators may have an overly simplistic view of eCNF morphology and measurement limitations, which influences their expectation as to the types of information requested for describing the eCNF material. The eCNFs diverge greatly from more traditional nanoparticle morphologies (e.g., spheres, rods, cubes, etc.), as they have overall 3D dimensions of 1 µm or more, but also have a hierarchical branched or network structure at the nanoscale (Fig. 2). Additionally, eCNF morphology, both external and branching nanostructure dimensions, changes depending on whether it is in the dry or wet state, and it may also be subject to artifacts from imaging sample preparation. Thus, regulators may request information that may be "simple" to measure for typical inorganic nanoparticle morphology without realizing the difficulty or irrelevance it has with regard to eCNF morphology.

The group identified several strengths and opportunities that could be used to address these key threats, which centered on revising CNF terminology/definitions, the need for improved SEM imaging, and building from past successes in satisfying requirements from regulators. Building from prior success in satisfying regulators, flexibility will be needed to address the different types of regulation requirements. FiberLean Techologies GmbH (Werhahn Group; Neuss, Germany) was able to successfully satisfy regulatory requirements for their CMF material for food contact and coating applications. Their approach was based on continuous consultation with regulators to understand what was needed and come up with the necessary measurements that the given regulatory agency agreed would be acceptable. This approach focused on filtration, mass fraction measurement, and high-resolution SEM. The results were benchmarked against existing products containing "traditional" forms of cellulose, allowing for side-by-side comparison that allowed regulators to assess if CMF was behaving in negative ways as compared to existing products.

The group also recommended that the definition of CNF needs to be refined to be more reflective of the complexities of this material. The eCNFs have hierarchical branching structures extending in three dimensions. The overall di-

mensions are 1 µm or more, but the internal branched or network structure is at the nanoscale, having a distribution of widths and length segments. It is not the typical fiber morphology that the nano-community is accustomed to (e.g., carbon nanotubes). Additionally, a new terminology should be developed that more realistically describes this material; ideally, the terminology should remove the word "nano" and opt for terms like "fibrillated cellulose" or "highly refined cellulose." However, even though eCNF may not be a nanosized object, it has a branched fibrillated nanostructure that is fully accessible to the environment, such that the resulting extremely high surface area-to-volume ratio would still need to be characterized and regulated.

WHAT IS NEEDED VS. WHAT IS FEASIBLE

The feasibility of a particle size measurement method depends on the context: is it for laboratory use or industrial use? Within the CNF industry, there are also different contexts, ranging from process control to R&D work similar to that carried out in universities and research labs. In industry, the work is generally internal and has less need for international standardization, except in the case of grade definition in patenting, and potentially in meeting safety and regulatory requirements. Larger companies may have sufficient resources to develop their own methods, if there is a wish to operate "in secret," while smaller companies may be dependent on ready-made documents available from ISO to guide their work.

When discussing feasibility, we need to consider measurement time, result quality, and resources needed. In many cases, the resources needed are proportional to the time required. When a large investment is needed, that proportionality is altered. A simplified view is that we have two variables: the measurement time and the result quality. In many cases (but not always), a long measurement time means a high-quality result and vice versa. A relatively slow measurement that gives a relatively low result quality is not going to be a feasible method when there are other options available.

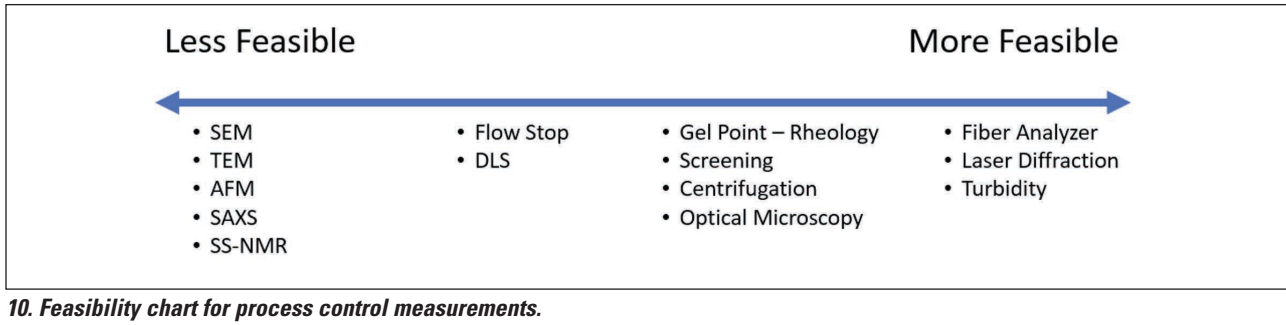
In the survey, we considered five motivations for measurement of CNF particle size: process control, product specification, benchmarking, safety/regulatory requirements, and grade definition for patents. Our later analyses show that this can be shortened to three main motivations, since benchmarking and grade definition could be seen as subcategories of product specifications.

Process control

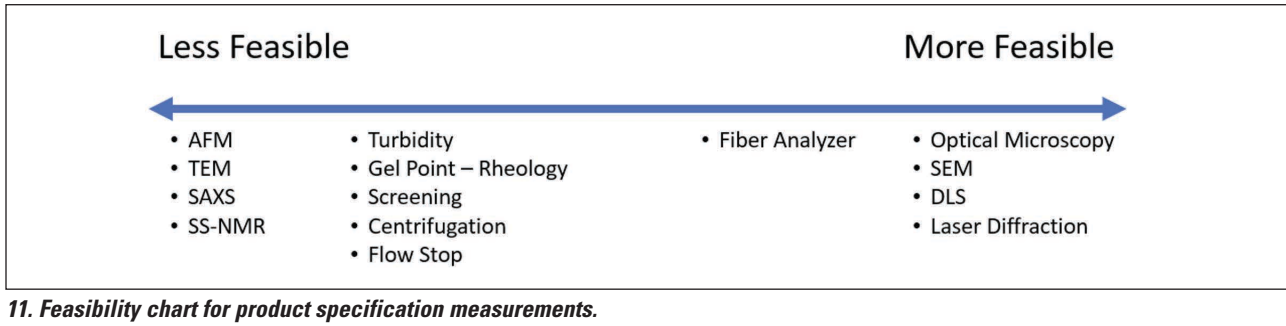
Process control demands fast measurement and is ideally performed inline (during production) without manual input. A lower accuracy is accepted as long as the repeatability is high. **Figure 10** shows a feasibility chart for process control. The recommended methods are image analysis by fiber analyzer, laser diffraction, and turbidity measurements. Depending on the size of the CNFs, the feasibility of each method will vary. Some companies producing CNFs at the pilot or commercial scale have likely developed their own indirect measurements for CNF morphology for quality monitoring and control, although they are keeping their measurement principles and instruments confidential.

Product specification

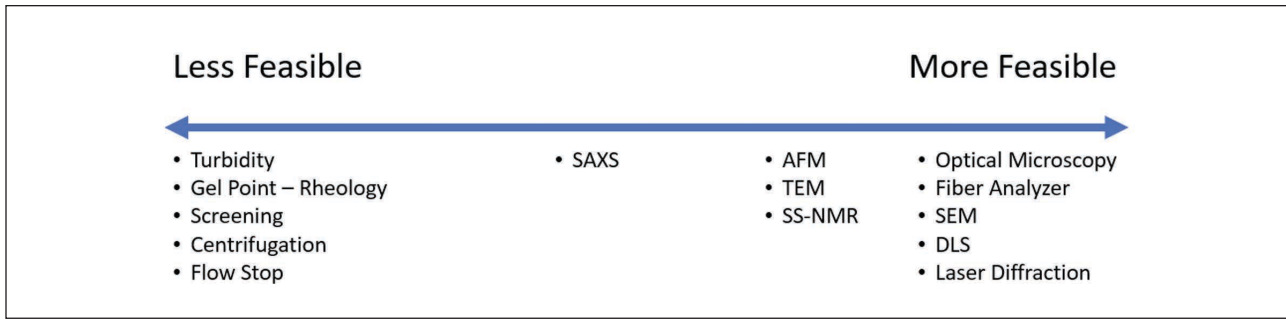
Product specification has less need for rapid measurements, as they are completed once per batch that is being shipped to a customer. **Figure 11** shows the feasibility chart for



10. Feasibility chart for process control measurements.



11. Feasibility chart for product specification measurements.



12. Feasibility chart for safety/regulatory measurements.

product specification. Here, more detailed information is needed, making the fiber analyzers and turbidity measurements less feasible than for process control. Instead, since a longer measurement time is accepted, DLS and optical microscopy become more feasible than for process control. The participants in the workshop preferred bulk measurement for product specifications above local imaging methods, but still thought that optical microscopy could be a nice complement. More complex methods (e.g., AFM, TEM, etc.) are still considered to be unfeasible, which was also confirmed by the workshop participants.

Safety/regulatory requirements

Meeting safety/regulatory requirements necessitates the highest quality information. Such detailed and accurate measurements are done even less often, perhaps as a part of a qualification process or for a petitioning process to obtain approval from a legal entity. The exact needs are case-dependent, but direct measurement of the fibrils is usually needed, often including the full particle size distribution, including the nanoscale portions. Both repeatability and accuracy are important. **Figure 12** shows the feasibility chart for safety/regulatory. Here, some complex methods, such as AFM, TEM, solid-state nuclear magnetic resonance (SS-NMR), and especially SEM, are feasible to use, but less complex methods such as DLS and laser diffraction can be useful in cases where the hydrodynamic radius gives sufficient information. Indirect methods are less feasible in the safety/regulatory context. The concern from the workshop participants about regulators having a simplistic view of eCNFs also emphasizes the need for images of fibrils and measurements of their true dimensions and branching features.

If we are to connect these findings with the survey results, **Table III** shows the most common CNF particle size measurement methods currently used by the industrial participants, grouped by measurement speed and the information obtained. It can be seen that only a few of the possible methods that were listed in Table I seem to be implemented in the CNF community. On the other hand, most of the methods that we concluded as "most feasible" in Figs. 10–12 are mentioned by the survey respondents. Thus, there seems to be agreement between the survey results and the workshop outcomes.

CONCLUSIONS AND THE PATH FORWARD

It was clear after the workshop that standard methods development can bring clarity to the complex topic of CNF size measurement, as well as education to those new to the field. However, there are still significant research questions that need to be solved. The standards can be incrementally improved as the science progresses. New research and international agreement on definitions will both be needed.

Possible ISO documents that can be developed

According to the survey results (Fig. 5), the three methods that were most widely used by industrial participants are SEM, laser diffraction, and image analysis using fiber analyzers. All these have good availability, and although SEM systems are expensive and require a vibration-free environment and at least one expert operator, this has not stopped

	Hydrodynamic Radius	Width, Length, Aspect Ratio
Slow	-	SEM, AFM, TEM (smaller size)
Medium	DLS (smaller size), Laser diffraction (medium size)	Fiber analyzers (larger size)
Fast	-	Online fiber analyzers (larger size)

DLS = dynamic light scattering; SEM = scanning electron microscopy; AFM = atomic force microscopy; TEM = transmission electron microscopy.

III. The methods used by survey respondents from industry, grouped by measurement speed and level of detail that can be measured.

companies from using it. For all three techniques, standard methods would be helpful. Table I shows that while there are some standard methods already, none have yet been adapted for eCNFs. Standards governing sample preparation will also need to be developed.

Workshop discussions focused more on wider topics rather than on individual methods. It appears the problem is bigger than measurement practicalities and that more fundamental questions need to be solved first. Recommendations from the discussions are listed as follows in what we believe should be their priority:

1. Define eCNF morphologies, including, for example, what is meant by the diameter of a (branched) cellulose nanofibril. The term "entangled cellulose nanofibril" should be defined. This could possibly become a part of ISO/TS 20477 (Nanotechnologies—Standard terms and their definition for cellulose nanomaterial).
2. Develop methods to reproducibly prepare samples, particularly eCNFs, for SEM and other high-resolution dry-imaging techniques.
3. Develop measurement guidelines for fibril diameter determination by dry imaging techniques, including SEM.
4. Adapt existing standard methods using DLS, laser diffraction, and automated fiber analysis for CNFs to develop methods for process control, including sample preparation and dispersion.

Research questions recommended for scientific work

There were a number of techniques discussed that require further refinement before they could be considered as a standard measurement approach. These are listed as follows:

5. Develop automated image analysis for eCNFs specific to each imaging technique (optical microscopy, AFM, TEM, and SEM).
6. Develop an approach to reproducibly prepare hierarchical branched CNF structures and characterize them. This will likely require the development of methods to prevent coalescence during drying. The "entanglement degree" could be studied and developed. Some kind of mathematical system or a scale would be needed to quantify a given object, and to be really useful, it should be possible to measure in the bulk, as an average or as a distribution.
7. The use of gel-point as an indicator of aspect ratio has found reasonable use; however, establishing the meaning and significance of gel-point when applied to eCNFs requires significant further work.
8. Develop a classification system for eCNFs encompassing measurements at multiple size ranges from micro- to nanoscale to fully characterize and distinguish eCNF samples. Such a classification system is likely to be critical for all standard method needs covered in this report.

It should be noted that many existing international standard methods are based on previous studies observing the shape of nanomaterials other than cellulose nanomaterials. For example, extensive work has been done to address the complexity of measuring irregular, polydisperse, and agglomerated nano-objects by TEM through a series of interlaboratory comparison studies on gold nanorods, colloidal silica, titanium dioxide nanoparticles, and carbon black [43-45]. The standard ISO 21363:2020 "Nanotechnologies—Measurements of particle size and shape distributions by transmission electron microscopy" is based on this work. This standard covers all stages of sample preparation, imaging, and data analysis (both qualitative and quantitative). A similar document, ISO 19749:2021 "Nanotechnologies — Measurements of particle size and shape distributions by scanning electron microscopy," has recently been published in ISO/TC 229. Despite dealing with very different nanomaterials, these documents would be a good starting point for CNF particle size standards development.

Some issues raised in the discussions are simply not mature enough for standardization. An analysis of Table III shows that there is a lack of rapid size measurement methods for the smaller size scales, regardless of detail level. One survey respondent wrote that "there is a lack of bulk measuring methods for the nanoscale," and the same opinion was voiced by some workshop participants. This would be a good area for method development.

The path forward

During 2023, TG 1 will establish teams to begin working on methods 1–4 and will be inviting collaborators and approaches for items 5–8. Anyone working in this space is welcome to collaborate, and formal membership in TG 1 is not required. Please contact Robert Moon (TG 1 convenor) at robert.j.moon@usda.gov or Colleen Walker (TG1 secretariat) at colleen.walker@maine.edu. **TJ**

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We chose to study this topic in order to help address the lack of standard methods for particle size characterization, which has hampered efforts at optimizing CNF production, quality control, and use in various applications. This report provides a general context as to why particle size measurements are needed for eCNFs, the difficulties and challenges of eCNF particle size measurement, and a summary of the findings from a survey of the CNF community and a workshop focused on "Addressing the gap: What is needed vs. what is feasible" in eCNF particle size measurement.

The most difficult aspect of this study was that it is unclear how to define CNF morphology in a way that enables researchers to know what features to measure. We did not solve this; rather, we highlighted what some of the challenges were to help educate the community.

Through this study, we found that we need to reconsider how we describe CNF materials. There is a global interest in solving the issue of how to describe CNF material and what features are needed to measure to best describe and characterize the particle morphology and size. If we can sort this out, it should aid in quality control assessments and grade definitions. The next step is to build dedicated teams of researchers and industry to jointly develop standards, technical specifications, and technical reports to address gaps in CNF particle size measurement approaches and protocols.

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ABOUT THIS PAPER

Cite this article as:
Moon, R., Hensdal, C., Beck, S., et al., *TAPPI J.* 22(2): 116(2023). https://doi.org/10.32964/TJ22.2.116

DOI: https://doi.org/10.32964/TJ22.2.116

ISSN: 0734-1415

Publisher: TAPPI Press

About this journal

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