

Fractional study of the microfibrillated cellulose

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ABSTRACT: The extremely high aspect ratios of minuscule lignocellulosic particles make analysis and characterization problematic. New methods to quantify the size, shape, and structure of cellulose microparticles are needed. The main objective of this research has been to study the potential of a novel fractional analysis method for microparticles. Laser diffraction-based particle size analysis was used as a reference method. Samples were also visualized using an electron microscope. Results of the novel fractional analysis method are presented as a percentage of particles in the different size fractions, according to the particle dimensions. The process is potentially a practical method for analyzing the morphological properties of microcellulose. Tube flow fractionation works especially well for particles with high aspect ratios, which typically pose a major problem for conventional particle analysis methods.

Application: This fractional analysis method works well for particles with a high aspect ratio, such as lignocellulose materials. It is potentially a practical method for analyzing the morphological properties of fine cellulosic particles.

Novel cellulose-based or cellulose-derived materials are potential raw materials on which to base a new biopolymer composites industry, produce green chemicals, rheologically modify paints and adhesives, or strengthen paper [1-5]. Microcellulose and nanocellulose also offer sustainable and viable alternatives to synthetic materials because cellulose is the most abundant renewable and biodegradable biopolymer on Earth [6-9]. These raw materials have outstanding chemimechanical properties, functionality, durability, and uniformity delivered by the hierarchical structure found in the particulars of macroscopic dimensions [10-12]. Extensive work on the fine structure of wood-derived cellulose has revealed constituent structures labeled by their smallest dimension as, for example, microfibrillated cellulose (MFC), nanofibrillated cellulose (NFC), microcrystalline cellulose (MCC), and nanocrystalline cellulose (NCC). Fine cellulosic particles have characteristic size, aspect ratio, morphology, crystallinity, and surface properties. Depending on their end use, cellulose microparticles are primarily classified by size and aspect ratio, describing whether the particles are microscale or nanoscale, and have flake-like planar point-like particles or fibrillar-shaped elongated strands.

Typically, cellulose-based microparticles are highly heterogeneous and have wide size distribution [10,12-15]. The shortest dimension of the particles may be nanoscale, while the longest dimension of the particles may be microscale. Wang et al. [16] revealed that nanofibrillated cellulose networks have two major structures. One is highly kinked, naturally helical, but not twisted fibrils, which form as backbones of nanofibrillated cellulose networks. The other type is “soft looking” fibrils that entangle the fibril network. These

fibrils are highly twisted and curled. The backbone and “soft looking” fibrils appear in different features of a nanofibrillated cellulose networks, such as “blooming tree,” “ghost,” or “tree,” “flower,” or “torch” [16]. Because of their high aspect ratios, cellulose-based microfibers are difficult to measure accurately using conventional particle analysis methods. In addition, to enable the industrial-scale production of desired micromaterials and nanomaterials, there is an increasing need for the development of measurement and characterization methods for small-scale particles [17].

To date, most promising image analysis methods have been largely based on direct observation by electron or atomic force microscopes, quantification by light scattering or laser diffraction methods, or indirect measurements of, for example, transmittance, viscosity, settling, or water retention values. Many of these methods are problematic when analysis is required to provide fast responses or quantify larger samples. In addition, results from several analysis methods need to be combined to gain a representative illustration of the sample properties. One major drawback is that scattering-based analyses commonly estimate the particles' spherical qualities, regardless of the actual shape of the particles under evaluation [18-20]. One promising way to overcome such drawbacks could be to fractionate large network fibrillated cellulose from the small ones using a centrifuge technique. Wang et al. showed that the centrifuging technique can statistically fractionate small nanofibril networks from large ones [16].

It seems evident that a better way to analyze the morphology of wood-based microparticles is needed. It is probable that legislation will mandate that different operators in the bio-economy value chain control the morphological characteristics of their raw materials and products in the near

future. To fill this gap, we have developed a fractional analysis method for characterization of elongated lignocellulose particles.

Our investigation presents a fractional particle characterization scheme based on particle size and shape profiling. Samples in a constant water flow were fractionated primarily by the longest dimension of the particles. This helps characterize particles in detail and determine their relative proportion in the pulp. The physically separated size fractions may be analyzed with image analysis software and various microscopic methods. Results of the analysis can be presented as a percentage of particles in the different fractions, according to the particle dimensions. Additionally, aspect ratios can be studied by image analysis of the fractions. Particle classification based on the tube flow fractionation principle works especially well for particles with high aspect ratios. The technique is also fast and easy to perform. In addition, the method has good repeatability.

MATERIALS AND METHODS

Pulps

This research used Celish KY-100G (Daicel Chemical Industries; Tokyo, Japan), a commercial MFC made from purified wood pulp. The dry matter content of the original MFC was 95%, and the average aspect ratio (AR) (ratio between longest and shortest dimension) of this MFC was analyzed to be more than 20, based on image analysis.

Methods

Fractionation by tube flow

Pulp particles were classified into four different size categories and monitored using a method based on the tube flow fractionation principle, with ionized water (25°C) acting as the mobile phase. **Figure 1** presents a schematic description of the tube flow fractionation procedure [21]. For this study, we used the same fractionation principle described by Laitinen [22], but with smaller fractionation equipment. Tubes used for the study were Teflon coated, and counter-pressure during fractionation was 2 bars.

A 5 mL sample at a consistency of 0.1% was fractionated with deionized water for 100 s at a flow rate of 7.7 mL/s in a long plastic tube (4 mm diameter) to separate the particles according to their size. Four different fraction (FR) size categories were obtained by taking the samples after separation at specific fractionation times (FR1 = 57-70 s, FR2 = 70-81 s, FR3 = 81-88 s, and FR4 = 88-100 s). The largest particles in the fractionated particle-water suspension were visualized with a high definition camera unit. During fractionation, approximately 1000 individual pictures were captured. **Figure 2** presents two examples of pictures of MFC samples captured during tube flow fractionation.

Image analysis uses the “sub-pixel algorithm,” which means that, in practice, image analysis measurement sensitivity is around 1 μm . The average AR of visible particles in the fractions – the relation of the longest dimensions (LD) and the

shortest dimensions (SD) – were obtained from optical image analysis (fractionation images from tube flow). The amount of material in the different size categories was measured by filtration on a membrane (0.1 μm retention) followed by weighing. Filtrations were performed with a filtration funnel (50 mm diameter). Filtration was performed with adequate amounts of commercial flocculent (cationic polyacrylamide [CPAM]) to achieve the highest possible retention.

Field-emission scanning electron microscopy

Field-emission scanning electron microscopy (FESEM) was used to visualize the microstructure of the different fractions of the samples. The FESEM samples were prepared by rapid freezing with liquid nitrogen, followed by freeze-drying in a vacuum overnight. The dried samples were sputter-coated with platinum. Low voltage (10 kV) and a working distance of around 3 mm were used when imaging the samples.

Laser diffraction particle size analysis

We used an LS 13 320 (Beckman Coulter; Brea, CA, USA) commercial laser diffraction particle size analyzer as a reference method. The sample passes through a laser light in a chamber, and the diffraction of the light caused by the particles was determined by a series of detectors. The particles scatter light at an angle that is inversely proportional to their size. Detectors measure the intensity of light as a function of angle, and that information is used to calculate the particle size distribution with a complex inversion matrix algorithm based on the Fraunhofer theory of light scattering. The optical models assume that the particles are spherical, and that the result is independent of how the light hits the rotating particle. The device is able to determine the size distribution of particles inside a range of 0.4-2000 μm using 134 detectors [18-20].

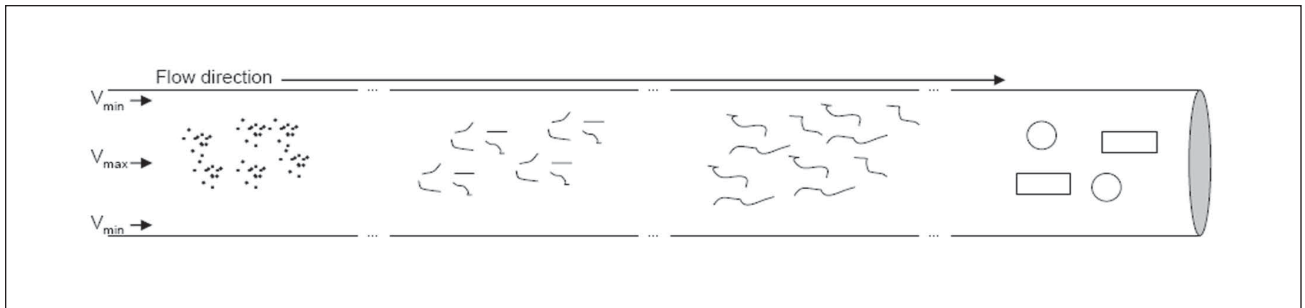
RESULTS AND DISCUSSION

This investigation presents a fractional particle characterization scheme based on particle size and shape profiling. Samples in a constant water flow were fractionated primarily by the longest dimension of the particles.

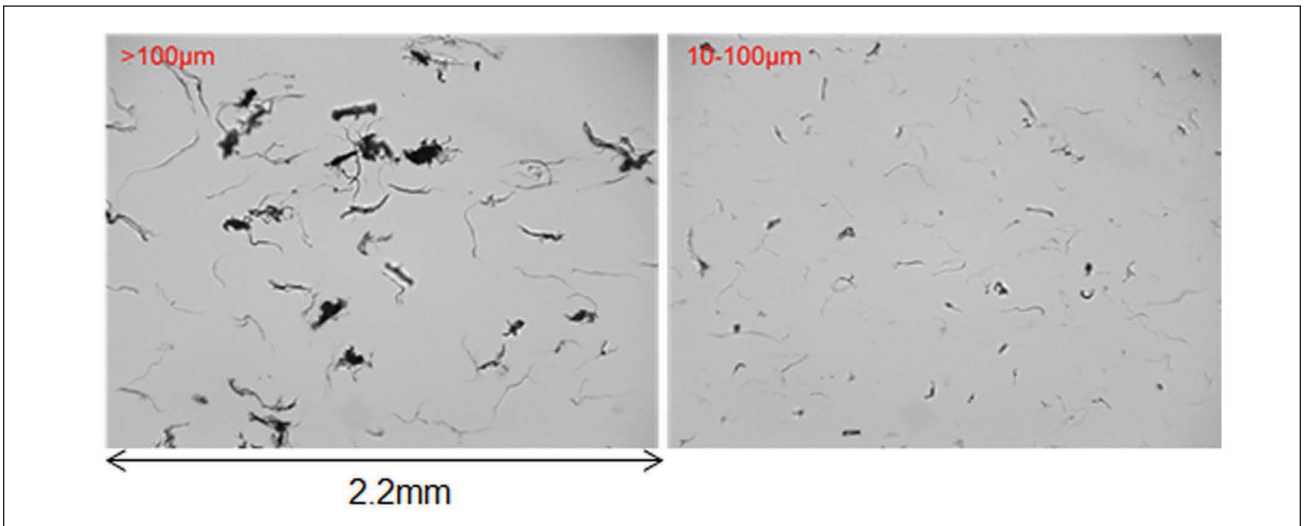
Repeatability of fractional analysis

Repeatability tests were performed using MFC to clarify the reliability of optical image analysis and determination of mass percentages. Mass percentages of different fractions were analyzed by weighing membranes after filtration. When filtrations were performed, flocculent (CPAM) was added to all fractions to achieve the highest retention possible. **Figure 3** presents 10 parallel mass percentage analyses of the MFC, with a 95% confidence level. Based on repeatability tests consisting of 10 parallel measurements, variation (i.e., two times standard deviation) of the combined mass percentages analysis with fractionation, filtration, and weighting was approximately 7%.

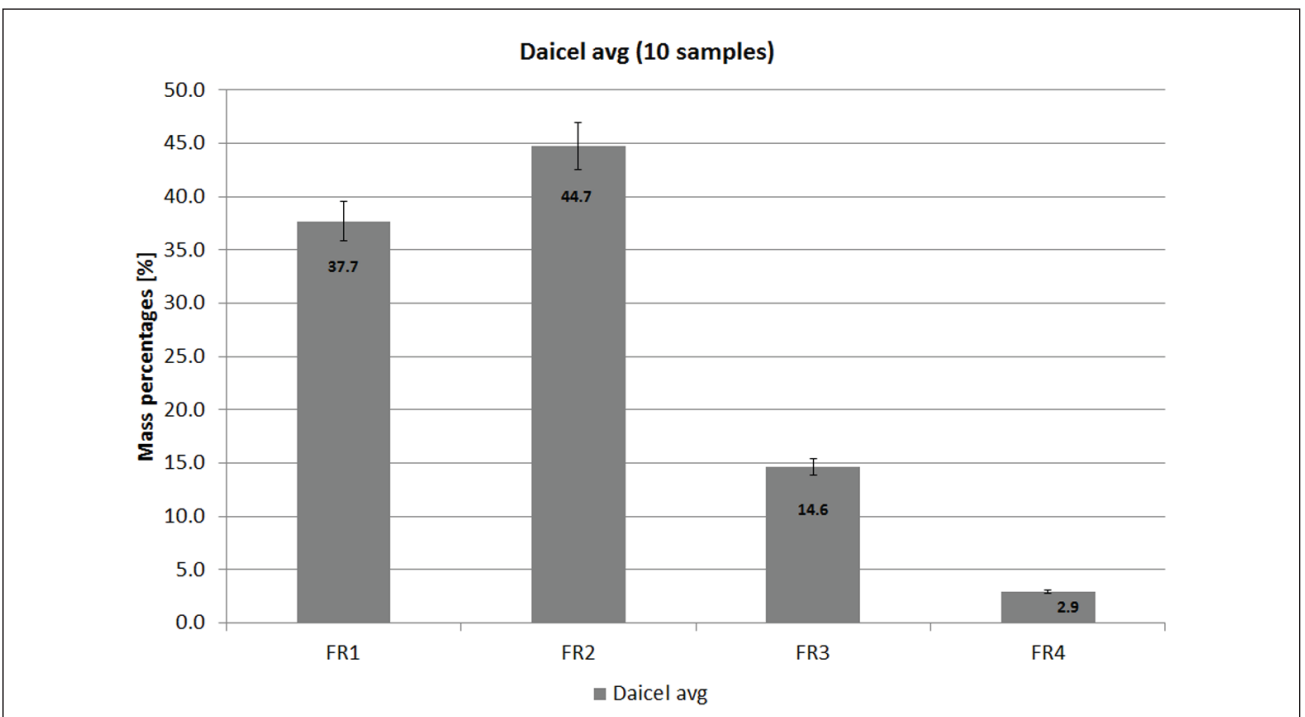
Figure 4 (a and b) presents 23 parallel optical image



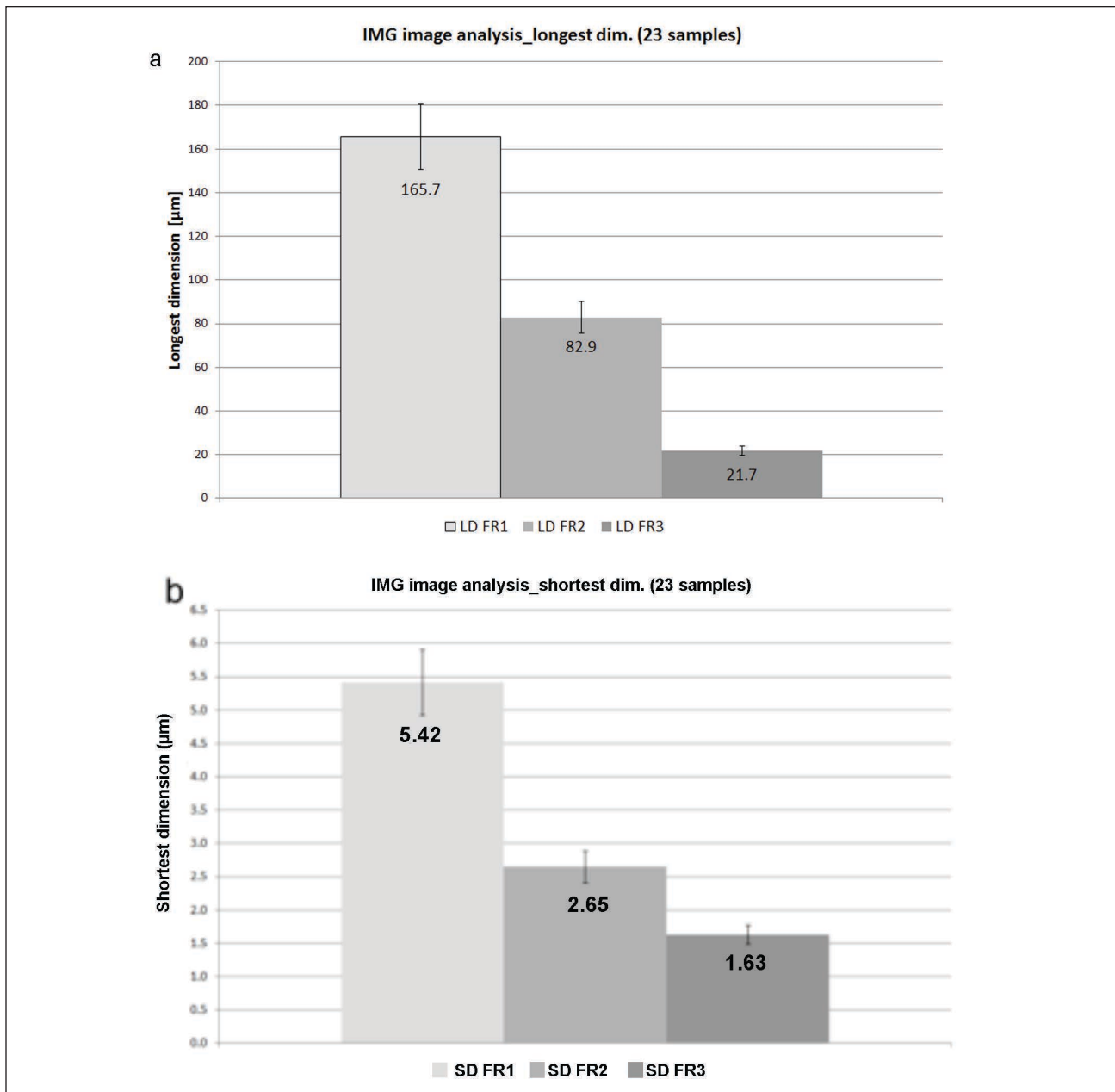
1. Fractionation principle [21].



2. Examples of individual optical images from fractions FR1 (left) and FR2 (right).



3. Mass percentages of different fractions with microfibrillated cellulose (MFC) (95% confidence level).



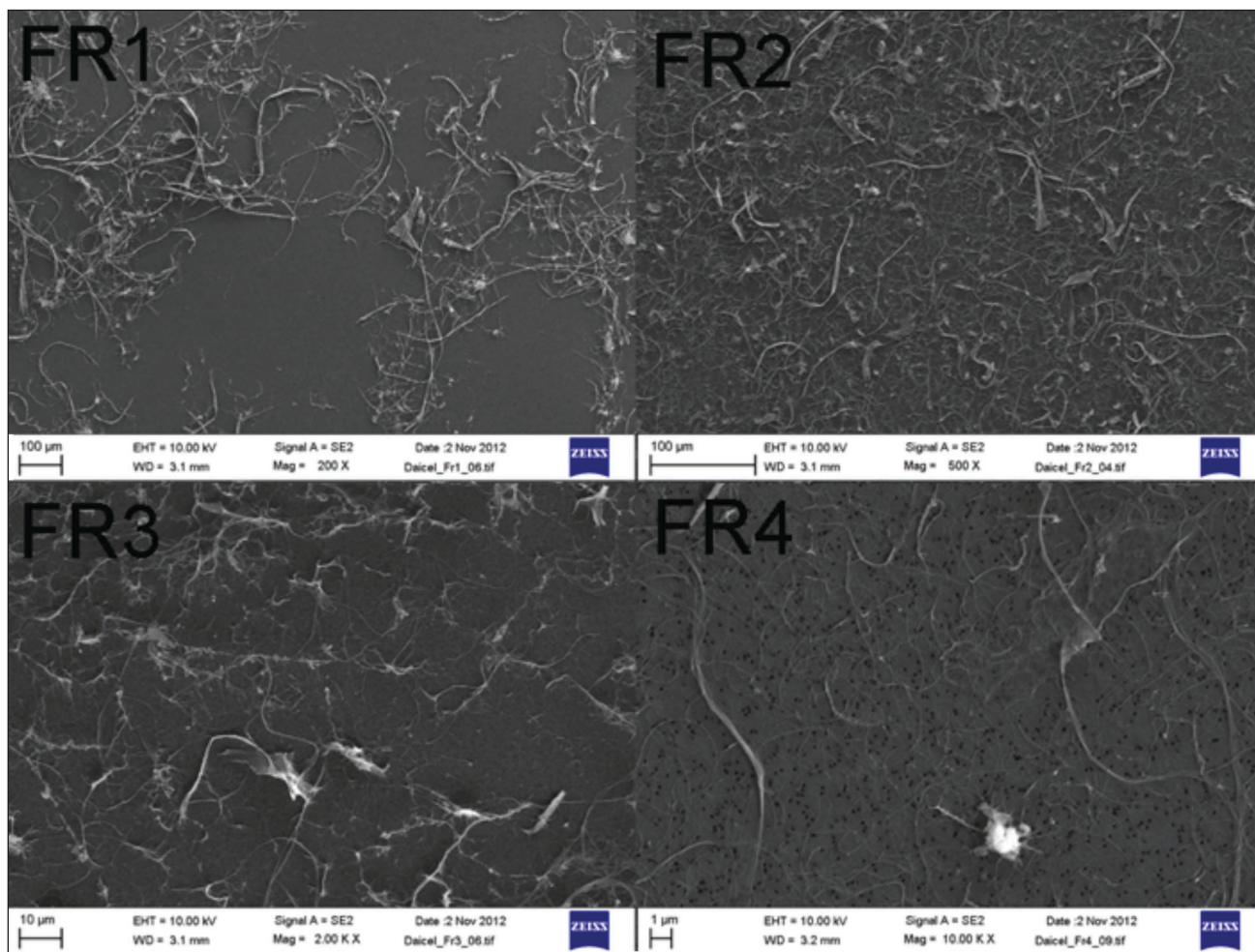
4. The longest (a) and shortest dimensions (b) of MFC pulp in different size categories (i.e., fractions) based on image analysis. Results based on 5.8 million analyzed particles.

analysis results (including sample preparation and fractionation) of the MFC, with a 95% confidence level for FR 1-3. Based on repeatability tests (consisting of 23 parallel measurements in each fraction and an average of 84000 particles for each), the variation (i.e., two times standard deviation) of the combined sample fractionation and optical image analysis was approximately 9%. The shortest dimensions of particles in FR4 are typically very small (much less than 1 μm). This means that optical microscopic methods cannot be used to analyze particle size. As shown in Figs. 4a and 4b, average particle sizes (LD and SD) decreased when moved on to smaller size fractions. Repeatability of image analysis was accept-

able. This ensures that it is possible to compare various pulp samples based on image analysis results.

Fractional analysis of MFC

Particles in the fractionated suspension were visualized with a charge-coupled device (CCD) camera, and FESEM was used to visualize MFC particles in the submicron range. **Figure 5** shows FESEM pictures of each fraction (i.e., size categories of Daicel). As seen from FESEM images of MFC pulp fractions (Fig. 5), the particles present show a typical fiber-like structure and considerable fibrillation. The particles in FR1 are long and thin, with their length being several hundred micrometers, thus



5. Field-emission scanning electron microscopy (FESEM) pictures of different fractions. Note that each fraction has been imaged at different magnifications because of the different size of particles after fractionation.

making the aspect ratios very high. A single FESEM image is not able to detect many individual particles, and those visualized have severe overlapping that makes automated image analysis troublesome as a means to characterize particle dimensions. Fractional analysis particularly helps when choosing certain magnitudes of FESEM images. Without size fractionation, particle lengths are very difficult to determine because of entanglements and branching of the nanofibers, which make it hard to identify the ends of individual nanofibers. Fractional analysis by tube flow fractionation provides useful information about microcellulose or nanocellulose samples. Four size categories (**Table I**) were obtained using fractional analysis. Table I presents mass percentages, LD, and average AR of pulp samples from different size categories. In this case, AR describes the ratio between the longest and the shortest dimension. Different types of particles can be classified according to dimension of particles. The particles in FR4 are typically very small (much less than 1 μm). This means that it is impossible to analyze particle size using optical microscopic methods. Instead, AR results in the case of FR4 have been categorized based on visualization of the several FESEM-pictures.

As shown, most of particles are elongated (i.e., $\text{AR} > 10$) in the case of MFC. The analysis method described here enables the comparison of various samples by giving information that is not easy to achieve using conventional methods.

Table II presents some of the key values of the MFC sample analyzed by the laser diffraction method (Beckman Coulter LS 13 320). Mean and median size values are presented from the unfractionated sample, as are four different size fractions (fractionated by the tube flow fractionation method). The MFC particle size measurement with laser diffraction shows the clear differences for feed, and a decreasing particle size ($\text{FR1} > \text{FR2} > \text{FR3} > \text{FR4}$) in separated fractions (Table II). This result ensured that the tube flow fractionation principle works well for the elongated particles. This also means that the fractionation method may be useful as a pretreatment for many commercial particle size analysis methods. When measuring particle size distributions, small particles in the investigated samples can become blocked by the larger ones; this results in a larger median particle size than in the actual sample. Clear differences between size categories (i.e., fractions) were observed, even if the absolute values of particle sizes could be

Fraction	Overall Statistic	Aspect Ratio >10	Aspect Ratio = 5-10	Aspect Ratio <5
FR1	37.7% mass 166±15 µm LD (average) 30.7 aspect ratio (average)	60% of particles	16% of particles	24% of particles
FR2	44.7% mass 83±7 µm LD (average) 30.7 aspect ratio (average)	60% of particles	20% of particles	20% of particles
FR3	14.6% mass 22±2 µm LD (average) 13.8 aspect ratio (average)	54% of particles	26% of particles	20% of particles
FR4	2.9% mass <10 µm LD (average) <1 µm SD (average)	55%-60% of particles	20%-25% of particles	15%-25% of particles

I. Analysis results of MFC pulp (Daicel Celish KY-100G). Different types of particles and their proportions in different fractions (FR). LD = longest direction, SD = shortest direction.

	Unfractionated	FR1	FR2	FR3	FR4
Mean, µm	66.2	84.8	31.4	8.4	0.7
Median, µm	35.9	52.2	18.9	6.7	0.3
Specific surface area, cm ² /mL	5635	3422	9193	36263	280000

II. Key values of the MFC sample analyzed by the laser diffraction method (including the unfractionated sample and different size fractions [FR]).

incorrect. Because laser diffraction particle size analysis is incapable of accounting for the aspect ratio of particles, caution must be taken when analyzing nonspherical particles using laser diffraction. In addition, Xu and Di Guida [18] pointed out that, in nonspherical particles with large aspect ratios, a laser diffraction measurement produces an oversized size distribution and exaggerates distribution broadness because of particle orientation effects and deviation from the spherical models used in data processing.

As with any new materials, proper characterization of microfibrillated and nanofibrillated celluloses is essential for their manufacture, trade, and safety assessment [16,17,23,24]. However, characterization of fibrillated celluloses is challenging because of their heterogeneous nature and because they mostly consist of long, slender fibrils with a high degree of branching. Direct characterization of fibrillated celluloses has relied primarily on microscopic imaging. However, manual microscopic evaluation is time-consuming and is based on subjective assessment of small areas in the larger sample. In addition, improper pretreatment can introduce artifacts into the images. Some indirect measurement methods, which generally look at the relationship to the particle size of fibrillated celluloses, have also been introduced. The main problem is still that very few methods have been

shown to work well for fibrillated cellulose. As an example, methods suitable for measuring the particle size distribution of (presumably) spherical particles, such as methods based on light scattering, are not generally well suited for high aspect ratio particles such as fibrillated celluloses.

CONCLUSIONS

The microfractionation method presents a very promising and practical method of analyzing morphological properties of microcellulose and nanocellulose. Fractionation works well, especially for particles with a high aspect ratio (such as lignocellulose). This group of particles typically poses a major problem for all other particle size measurement methods. This study has shown that repeatability of the equipment is at an acceptable level. Additionally, the device forms an analysis platform that enables the combination of several measurement principles (e.g., image analysis [visible particles], various optical signals, and flow fractionation information). After the analysis platform is further improved, the results could be automatically presented as a percentage of different particles in the different size fractions. The method is very promising, but work is still needed to obtain reliable information on the smallest particles (much less than 1 µm). **TJ**

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ABOUT THE AUTHORS

We chose this topic to research because new methods to quantify the size, shape, and structure of cellulose microparticles are needed. This study is a supported by more than 10 years' experience in the use of fractional analysis.

This study demonstrated that the fractionation principle works well for the elongated particles. Once the analysis platform is further improved, the results could be automatically presented as a percentage of different particles in the different size fractions.

Microcellulose particles are challenging to measure reliably with conventional particle analysis methods because of their extremely high aspect ratios. Mills can use the fractional analysis method to compare various samples and obtain information that is not easy to achieve using conventional methods.



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