

A dynamic characterization method for micro-nanocellulose

Technical field

The invention relates to the field of characterization of nanocellulose from flexible materials, in particular to characterization methods of plant-
5 derived micro-nanocellulose materials.

Background art

Micro-nanocellulose has excellent properties which is environmentally friendly, natural and recyclable, biocompatible, and has excellent optical and mechanical properties. At present, micro-
10 nanocellulose materials have been applied in various fields, such as cosmetics, biomedicine, construction, food, military, paper, environmental protection, etc., and have great prospects.

Current characterization methods for micro-nanocellulose include TEM/SEM, AFM (atomic force microscopy), and DLS (dynamic light
15 scattering), etc. The existing methods are time consuming, complicated in preparation, and difficult to operate.

Summary of the invention

The main object of the present invention is to overcome the shortcomings and deficiencies of the prior art, and to provide A dynamic
20 characterization method for micro-nanocellulose, which uses the existing particle tracking velocity measuring technique to capture nanocellulose in a microfluidic channel observed under a microscope by a CCD camera.

Then parameters such as length, diameter, speed and quantity of the nanocellulose may be obtained by analyzing data derived from the image captured by the CCD camera.

In order to achieve the above object, the present invention provides
5 the following technical solutions:

A dynamic characterization method for micro-nanocellulose comprises the following steps:

(1) intermittently sonicating a micro-nanocellulose suspension for 5-10min with an interval of 3s;

10 (2) injecting the micro-nanocellulose suspension treated by step (1) to a micro-nano scale microfluidic channel by a micro-injector;

(3) adjusting an objective lens of a microscope to ensure the microfluidic channel is in within the field of view of the microscope and exhibits a clear image, then observing how the micro-nanocellulose flows
15 in the microfluidic channel by the microscope to confirm that the micro-nanocellulose is flowing in the channel at a suitable velocity;

(4) using a microscope directly connected CCD camera to capture the micro-nanocellulose in the microfluidic channel, and keeping all positions unchanged during the capture to reduce errors; after the capture, moving a
20 stage to measure the parameters of the micro-nanocellulose in the microfluidic channel at different positions;

(5) transmitting photos taken by the CCD camera to a computer for

image data processing, and distinguishing nanocellulose from water according to different pixel gray values of the nanocellulose and the water on an image; calculating the length and diameter of the nanocellulose by plotting a minimum circumscribed rectangle and an inscribed circle based
5 on the micro-nanocellulose profile; calculating the velocity of the nanocellulose by center point displacement of the nanocellulose in multiple frames; and calculating the quantity of the nanocellulose by counting the amount of nanocellulose flowing through the system for a certain period.

Preferably, step (1) is specifically as follows:

10 intermittently sonicating the 0.1% micro-nanocellulose suspension for 10 min with a mode of sonication for 3s followed by an interval of 3s at a power of 300 W and a temperature of 0-4 °C.

Preferably, in step (3), said suitable velocity is 0-200um/s, and if the velocity is beyond the range, the observation is not conducted until the
15 velocity is lowered.

Preferably, in step (4), said parameters of the micro-nanocellulose in the microfluidic channel measured by moving a stage at different positions comprises length, diameter, velocity and quantity of the nanocellulose.

Preferably, step (5) further comprises identification of the micro-
20 nanocellulose with the following process:

when the micro-nanocellulose move in the field of view, in consecutive frames, the micro-nanocellulose move continuously along the

flow direction, while the optical properties of the suspension and the background during the observation are stable without change, so that in a plurality of the consecutive frames or even in the entire video, the imaging of the background on the CCD is invariant, and moving nanocellulose in the video is identified; wherein the micro-nanocellulose in the micro-nanocellulose suspension are irradiated by a light source to produce weak scattered light, and after imaging on a CCD camera, the micro-nanocellulose has a different profile from the background with a brighter center and darker edge.

Preferably, in step (5) length and diameter of the nanocellulose is calculated by:

in one frame of the image, identifying the nanocellulose by the background difference: based on the gray value difference between the nanocellulose and the background, drawing a profile of the nanocellulose with a gray value threshold, confirming the profile of the nanocellulose in the frame, and drawing a minimum circumscribed rectangle on the nanocellulose profile to enclose the micro-nanocellulose, so that the long side of the rectangle is the length of the micro-nanocellulose, and the length is recorded in units of pixels; and drawing an inscribed circle within the profile of the nanocellulose, so that the diameter of the inscribed circle is the diameter of the nanocellulose, and the diameter is recorded in units of pixels; and

according to the size of the field of view determined by the magnification of the microscope, and the resolution of the CCD, determining the actual length of one pixel in the image, thereby converting the pixel unit into a length unit.

5 Preferably, in step (5) velocity of the micro-nanocellulose is calculated by:

identifying a nanocellulose, determining the center point position of the imaging profile of the nanocellulose, and analyzing the displacement of the center point of the nanocellulose in two consecutive frames, thereby
10 obtaining the displacement of the center point of the nanocellulose in certain time interval; then calculating the velocity of the nanocellulose at the moment by $V=S/t$, and in a stable flow, calculating an average velocity of the nanocellulose by continuous analysis of multiple frames.

Preferably, in step (5) the quantity of the micro-nanocellulose is
15 calculated by:

given that the nanocellulose flows in from one side of the field of view in the flowing liquid and flows out from the other side, treating the frame where nanocellulose is identified by the system at the edge of the flow-in side of the nanocellulose in the field of view as a first frame, identifying
20 and tracking each subsequent frame until the nanocellulose disappears on the other side of the field of view, wherein the nanocellulose is counted as 1; then repeating the process to identify and track multiple targets

simultaneously so that a certain amount of nanocellulose is observed in a certain period of time, and labeling the cellulose in the order in which the fibers are identified by the system automatically; wherein the labeling method is specifically: labeling the nanocellulose sequentially entered in the field of view as 1, 2, 3, 4...n, respectively, so that starting from 1 till the end of the video, the label of the cellulose indicated by the system is the number of nanocellulose identified in the video.

Compared with the prior art, the present invention has the following advantages:

1. The invention can perform real-time dynamic tracking and measurement on micro-nanocellulose with short measuring time and large amount of processed information. It can not only measure the length and diameter of nanocellulose, but also velocity and position change of nanocellulose in the flow field; the prior methods such as SEM/TEM/AFM measure size of the solid nanocellulose statically, and the nanocellulose needs to be dried before the measurement, therefore the existing measurement for nanocellulose is time consuming and complicated in operation. Compared with the existing methods, the present invention has the advantages of short time, simple operation, and availability of multiple parameters at the same time.

2. The present invention can count the distribution of parameters such as number, length, and diameter of nanocellulose in the channel, which is

not possible in the prior art.

Brief description of the drawings

Fig. 1 is a flow chart of a dynamic characterization method for micro-nanocellulose according to the present invention;

5 Fig. 2 shows the effect of irradiation by a light source to nanocellulose according to the present invention;

Fig. 3 shows a circumscribed rectangle and an inscribed circle of the profile of a nanocellulose according to the present invention;

Fig. 4 is a schematic structural view of an experimental apparatus of
10 Example 2;

Fig. 5 shows nanocellulose of Example 2 in a channel;

Fig. 6 is a statistical diagram of the length of nanocellulose in
Example 2;

Fig. 7 is a statistical diagram of the width of nanocellulose in Example
15 2;

Fig. 8 is a statistical diagram of the velocity of nanocellulose in
Example 2.

Embodiments

The present invention will be further described in detail below with
20 reference to the embodiments. The advantages and features of the present invention are more readily understood by those skilled in the art, so that the scope of the present invention is more clearly defined. It is understood

that the specific embodiments described herein are merely illustrative of the invention and are not intended to limit the invention.

Example 1

As shown in Fig. 1, a dynamic characterization method for micro-nanocellulose of Example 1 comprises the following steps:

(1) intermittently sonicating a 0.1% micro-nanocellulose suspension for 30min with a mode of sonication for 3s followed by an interval of 3s at a power of 300 W and a temperature of 0-4 °C;

(2) injecting the micro-nanocellulose suspension treated by step (1) to a micro-nano scale microfluidic channel by a mirco-injector;

(3) adjusting an objective lens of a microscope to ensure the microfluidic channel is in within the field of view of the microscope and exhibits a clear image, then observing how the micro-nanocellulose flows in the microfluidic channel by the microscope to confirm that the micro-nanocellulose is flowing in the channel at a suitable velocity, wherein said suitable velocity is 0-200um/s, and if the velocity is beyond the range, the observation is not conducted until the velocity is lowered;

(4) using a microscope directly connected CCD camera to capture the micro-nanocellulose in the microfluidic channel, and keeping all positions unchanged during the capture to reduce errors; after the capture, moving a stage to measure the parameters of the micro-nanocellulose in the microfluidic channel at different positions;

(5) transmitting photos taken by the CCD camera to a computer for image data processing, and distinguishing nanocellulose from water according to different pixel gray values of the nanocellulose and the water on an image; calculating the length and diameter of the nanocellulose by plotting a minimum circumscribed rectangle and an inscribed circle based on the micro-nanocellulose profile; calculating the velocity of the nanocellulose by center point displacement of the nanocellulose in multiple frames; and calculating the quantity of the nanocellulose by counting the amount of nanocellulose flowing through the system for a certain period.

step (5) further comprises identification of the micro-nanocellulose with the following process:

when the micro-nanocellulose move in the field of view, in consecutive frames, the micro-nanocellulose move continuously along the flow direction, while the optical properties of the suspension and the background during the observation are stable without change, so that in a plurality of the consecutive frames or even in the entire video, the imaging of the background on the CCD is invariant, and moving nanocellulose in the video is identified; wherein the micro-nanocellulose in the micro-nanocellulose suspension are irradiated by a light source to produce weak scattered light as shown in Fig. 2, and after imaging on a CCD camera, the micro-nanocellulose has a different profile from the background with a brighter center and darker edge.

Length and diameter of the nanocellulose is calculated by:

in one frame of the image, identifying the nanocellulose by the background difference: based on the gray value difference between the nanocellulose and the background, drawing a profile of the nanocellulose with a gray value threshold as shown in Fig. 3. For the convenience of observation, the image is magnified for several times so that the pixel is clear.

Then the profile of the nanocellulose in the frame is confirmed, and as shown in Fig. 3, a minimum circumscribed rectangle and an inscribed circle within the profile of the nanocellulose are drawn to figure out the length and diameter of the nanocellulose.

The inscribed circle is a circle that is inscribed in the blue profile whose diameter is equal to that of the nanocellulose. The size information is first recorded in pixel unit. The diameter of the inscribed circle is approximately 3 pixels (px).

The circumscribed rectangle, as shown in Fig. 3, is a minimum rectangle around the nanocellulose and envelopes the same, such that the long side of the rectangle equals to the length of the nanocellulose, which is about 17 pixels (px).

According to the size of the field of view determined by the magnification of the microscope, and the resolution of the CCD, the actual length of one pixel in the image may be determined, thereby converting the

pixel unit into a length unit.

Velocity of the micro-nanocellulose is calculated by:

identifying a nanocellulose, determining the center point position of the imaging profile of the nanocellulose, and analyzing the displacement of the center point of the nanocellulose in two consecutive frames, thereby
5 obtaining the displacement of the center point of the nanocellulose in certain time interval; then calculating the velocity of the nanocellulose at the moment by $V=S/t$, and in a stable flow, calculating an average velocity of the nanocellulose by continuous analysis of multiple frames.

10 Quantity of the micro-nanocellulose is calculated by:

given that the nanocellulose flows in from one side of the field of view in the flowing liquid and flows out from the other side, treating the frame where nanocellulose is identified by the system at the edge of the flow-in side of the nanocellulose in the field of view as a first frame, identifying
15 and tracking each subsequent frame until the nanocellulose disappears on the other side of the field of view, wherein the nanocellulose is counted as 1; then repeating the process to identify and track multiple targets simultaneously so that a certain amount of nanocellulose is observed in a certain period of time, and labeling the cellulose in the order in which the
20 fibers are identified by the system automatically; wherein the labeling method is specifically: labeling the nanocellulose sequentially entered in the field of view as 1, 2, 3, 4...n, respectively, so that starting from 1 till the

end of the video, the label of the cellulose indicated by the system is the number of nanocellulose identified in the video.

By the abovementioned steps, dynamic characterization of nanocellulose can be done by the present invention.

5 Example 2

The dynamic characterization method for nanocellulose of the present invention is carried out by the following specific experiments:

As shown in Fig.4, the instruments used in this experiment are:

Instruments	Model
Work station	Lenovo P410 (Think station) work station
Air floating isolation platform	ZPT-F-M-20-12
Microscope	Japan Olympus IX73 inverted microscope
Imaging unit	DU-897U-CS0-#BV of ANDOR, Oxford, UK

10 First, a 0.1% NFC (cellulose nanofibril) suspension was prepared, and then the NFC suspension was subjected to intermittent sonication for 30 min with a mode of sonication for 3s followed by an interval of 3s at a power of 300 W and a temperature of 0-4 °C, then the micro-nanocellulose suspension treated by the sonication is injected to a micro-nano scale microfluidic channel by a mirco-injector.

15 The light source of the microscope is provided by a power module TH4-200. When the power is on, black switch below the panel is closed.

A light source power switch and an adjustment switch of the microscope are located in front of the base, wherein the light source switch is on the left side, and the light source adjustment switch is on the right side. After the light source is turned on, the intensity of the light source is
5 adjusted in real time according to the real situation.

The position of the stage in XY axis direction is then adjusted so that the microfluidic channel is aligned with the objective lens during observation.

The knob below the microscope is a focus adjustment knob, where an
10 outer large knob is for coarse adjustment, and an inner small knob is for fine adjustment. The distance (focal length) between an objective lens and the microfluidic channel is adjusted to achieve a best observation distance.

The dial switch above the microscope is a splitter switch. When it is on the far left side, all the light is entered into an eyepiece for manual
15 observation. When it is in the middle, half of the light propagates to the eyepiece and half propagates to a computer imaging system. When it is on the far right side, then all the light propagates into the computer imaging system, which cannot be observed at the eyepiece.

At the beginning of the experiment, a 0.1% micro-nanocellulose
20 suspension is intermittently sonicated for 30min with a mode of sonication for 3s followed by an interval of 3s at a power of 300 W and a temperature of 0-4 °C. The sonicated NFC suspension is slowly injected into a

microfluidic channel. Since a high magnification objective lens is an oil lens, it is necessary to first drop a special oil on the lens, search for the channel under a low magnification objective lens, and move the target image under the low magnification lens to the center of the field of view followed by switching to the high magnification objective lens and Fine-tuning the focus until the image under high magnification is clear as shown in Fig. 5. When the NFC suspension flows to a portion of the channel which can be observed by the inverted microscope, the field of view under the microscope is recorded by the imaging unit on the computer workstation, and calculation is continuously conducted according to the video. Since no subsequent injection is performed, velocity of the nanocellulose in the channel will decrease continuously due to the viscous force of the inner wall of the channel. When the velocity in the field of view drops to about 0-200 $\mu\text{m/s}$, calculation is conducted according to the video with certain algorithm so that parameters such as quantity, length, diameter and velocity of the nanocellulose are obtained, as shown in Fig. 6, Fig. 7, and Fig. 8. Fig. 6 shows a length distribution of the nanocellulose in a range of 600-2000 nm, wherein nanocellulose with a length of 600-800 nm has the largest quantity, and that with a length of 800-1000 nm has the second largest quantity. Fig. 7 shows a diameter distribution of the nanocellulose, wherein the nanocellulose with a diameter of 600-650 nm has the largest quantity. Fig. 8 shows a velocity distribution of the nanocellulose in the

range of 0-80 $\mu\text{m/s}$, wherein nanocelluloses with a velocity of 20-30 $\mu\text{m/s}$ has the largest quantity.

The above embodiments are preferred embodiments of the present invention, but the embodiments of the present invention are not limited thereto, and any other changes, modifications, substitutions, combinations, and simplifications thereof made without departing from the spirit and scope of the invention should all be equivalent replacements and are included in the scope of the present invention.

1. A dynamic characterization method for micro-nanocellulose, characterized in comprising the following steps:

(1) intermittently sonicating a micro-nanocellulose suspension for 5-10min with an interval of 3s;

5 (2) injecting the micro-nanocellulose suspension treated by step (1) to a micro-nano scale microfluidic channel by a micro-injector;

(3) adjusting an objective lens of a microscope to ensure the microfluidic channel is in within the field of view of the microscope and exhibits a clear image, then observing how the micro-nanocellulose flows
10 in the microfluidic channel by the microscope to confirm that the micro-nanocellulose is flowing in the channel at a suitable velocity;

(4) using a microscope directly connected CCD camera to capture the micro-nanocellulose in the microfluidic channel, and keeping all positions unchanged during the capture to reduce errors; after the capture, moving a
15 stage to measure the parameters of the micro-nanocellulose in the microfluidic channel at different positions;

(5) transmitting photos taken by the CCD camera to a computer for image data processing, and distinguishing nanocellulose from water according to different pixel gray values of the nanocellulose and the water
20 on an image; calculating the length and diameter of the nanocellulose by plotting a minimum circumscribed rectangle and an inscribed circle based on the micro-nanocellulose profile; calculating the velocity of the

nanocellulose by center point displacement of the nanocellulose in multiple frames; and calculating the quantity of the nanocellulose by counting the amount of nanocellulose flowing through the system for a certain period.

2. The dynamic characterization method for micro-nanocellulose
5 according to claim 1, characterized in that, step (1) is specifically as follows:

intermittently sonicating the 0.1% micro-nanocellulose suspension for 10 min with a mode of sonication for 3s followed by an interval of 3s at a power of 300 W and a temperature of 0-4 °C.

10 3. The dynamic characterization method for micro-nanocellulose according to claim 1, characterized in that, in step (3), said suitable velocity is 0-200um/s, and if the velocity is beyond the range, the observation is not conducted until the velocity is lowered.

4. The dynamic characterization method for micro-nanocellulose
15 according to claim 1, characterized in that, in step (4), said parameters of the micro-nanocellulose in the microfluidic channel measured by moving a stage at different positions comprises length, diameter, velocity and quantity of the nanocellulose.

5. The dynamic characterization method for micro-nanocellulose
20 according to claim 1, characterized in that, step (5) further comprises identification of the micro-nanocellulose with the following process:

when the micro-nanocellulose move in the field of view, in

consecutive frames, the micro-nanocellulose move continuously along the flow direction, while the optical properties of the suspension and the background during the observation are stable without change, so that in a plurality of the consecutive frames or even in the entire video, the imaging of the background on the CCD is invariant, and moving nanocellulose in the video is identified; wherein the micro-nanocellulose in the micro-nanocellulose suspension are irradiated by a light source to produce weak scattered light, and after imaging on a CCD camera, the micro-nanocellulose has a different profile from the background with a brighter center and darker edge.

6. The dynamic characterization method for micro-nanocellulose according to claim 1, characterized in that, in step (5) length and diameter of the nanocellulose is calculated by:

in one frame of the image, identifying the nanocellulose by the background difference: based on the gray value difference between the nanocellulose and the background, drawing a profile of the nanocellulose with a gray value threshold, confirming the profile of the nanocellulose in the frame, and drawing a minimum circumscribed rectangle on the nanocellulose profile to enclose the micro-nanocellulose, so that the long side of the rectangle is the length of the micro-nanocellulose, and the length is recorded in units of pixels; and drawing an inscribed circle within the profile of the nanocellulose, so that the diameter of the inscribed circle is

the diameter of the nanocellulose, and the diameter is recorded in units of pixels; and

according to the size of the field of view determined by the magnification of the microscope, and the resolution of the CCD,
5 determining the actual length of one pixel in the image, thereby converting the pixel unit into a length unit.

7. The dynamic characterization method for micro-nanocellulose according to claim 1, characterized in that, in step (5) velocity of the micro-nanocellulose is calculated by:

10 identifying a nanocellulose, determining the center point position of the imaging profile of the nanocellulose, and analyzing the displacement of the center point of the nanocellulose in two consecutive frames, thereby obtaining the displacement of the center point of the nanocellulose in certain time interval; then calculating the velocity of the nanocellulose at
15 the moment by $V=S/t$, and in a stable flow, calculating an average velocity of the nanocellulose by continuous analysis of multiple frames.

8. The dynamic characterization method for micro-nanocellulose according to claim 1, characterized in that, in step (5) the quantity of the micro-nanocellulose is calculated by:

20 given that the nanocellulose flows in from one side of the field of view in the flowing liquid and flows out from the other side, treating the frame where nanocellulose is identified by the system at the edge of the flow-in

side of the nanocellulose in the field of view as a first frame, identifying and tracking each subsequent frame until the nanocellulose disappears on the other side of the field of view, wherein the nanocellulose is counted as 1; then repeating the process to identify and track multiple targets
5 simultaneously so that a certain amount of nanocellulose is observed in a certain period of time, and labeling the cellulose in the order in which the fibers are identified by the system automatically; wherein the labeling method is specifically: labeling the nanocellulose sequentially entered in the field of view as 1, 2, 3, 4...n, respectively, so that starting from 1 till the
10 end of the video, the label of the cellulose indicated by the system is the number of nanocellulose identified in the video.