**Replies to Editorial comments:**

The manuscript has been modified by the Science Editor to comply with the JoVE formatting standard. Please maintain the current formatting throughout the manuscript. The updated manuscript (55079\_R2\_062816.docx) is located in your Editorial Manager account. In the revised PDF submission, there is a hyperlink for downloading the .docx file. Please download the .docx file and use this updated version for any future revisions.  
  
1. Please combine all panels of one figure into a single image file.  
  
Please combine the a figures from Figure 1 into one single image file. Please combine the b images from Figure 1 into one single image file. This would create two figures for clarity. Alternatively, you can combines 1a1 and 1b1 into a side-by-side image file, etc.  
  
Despite your rebuttals and statements otherwise, the image files have not changed in the last two revisions.  
**RESPONSE:** We did made into two Figures. The label Fig. 1a1 and 1b1 do not exist anymore. The editor must read the old version. However, we loaded the individual images, such as 1a, 1b, 1c, 1d for the consideration to preserve the resolution of the Figure. I am sure the Journal has better software to combine these individual images into one Figure.

2. Please employ professional copy-editing services. Despite statements otherwise, the language is still very difficult and awkward to read and understand.  
**RESPONSE:** Proof read by our lab editorial service again.

3. Formatting:  
-Figures – For all multipanel figures, the panels should appear in a single file, on a single page, with all panels clearly labeled.  
**RESPONSE:** This is usually done by the Journal, not by the authors. Because Journal has better software to preserve resolution. We combined in the revised version per request.

-Please define all abbreviations at first occurrence (ie, AFM, etc.).  
**RESPONSE:** Done

-For all equipment, please include the company and product number in the materials table.  
**RESPONSE:** Very strange requirement. But done per request.

-9.1 – Please use “-“ instead of “~” to indicate a range.  
**RESPONSE:** Done

4. Please copyedit the manuscript for numerous grammatical errors, some of which are indicated below. Such editing is required prior to acceptance and should be performed by a native English speaker.  
-4.1 – “after separated the CNC dispersion”  
-Line 255 – “charge measured by zeta potential were”  
-Line 258 – “Thermal stability of the CNC and CNF samples were”  
-Line 263 – “Critical steps on the protocol”  
-Line 267 – “that the resultant CNC and CNF are thermally stable and the minimal loss of cellulose” – what about the minimal loss of cellulose?  
**RESPONSE:** Done

5. Additional detail is required:  
-2.9 – Are the fibers dried in the lab? If so, please describe the process.

**RESPONSE:** The editor must read the old version, No. 2.9

-3.1 – How much water is used? Which filtered solids? Is this the hydrolyzed pulp or hydrolysate?

**RESPONSE:** This is not critical. When we say wash, it usually 10:1 dilution. Depending what washing method is used. The amount of water usage are different.

-3.3 – What is the molecular weight cutoff?

**RESPONSE:** Dialysis bags are commercially purchased.

-4.1 – How is this done? Is the FCSR dried first? If so, how?  
**RESPONSE:** This is clearly described. Of course one need to dry to determine solids. We modified to reflect this comment.

-5.1 – How much is placed on the mica substrate?

**RESPONSE:** Just a droplet (amended). Any person skills in AFM will know this

-5.2 – How are the measurements/analyses performed? Please provide stepwise detail if this is to be filmed in detail or a citation if this is not to be filmed in detail.  
**RESPONSE:** This is not necessary. This paper is not about how to do AFM. Exact operation procedures differs for different AFM systems

-6.1, 9.1 – How is this done? Please provide a citation.  
**RESPONSE:** This is not necessary. This paper is not about how to do FTIR and X-Ray diffraction. These are standard lab equipment.

-8.1.2 – How is weight determined?  
**RESPONSE:** It is automatically determined by the instrument.

6. Branding: 5.2 – Image-Pro Plus

**RESPONSE:** Not really, we added the vendor name in revision.

7. Discussion: Please discuss the critical steps in more detail. Please discuss the limitations and future applications of the protocol. Please also discuss any troubleshooting/modifications that can be performed.

**RESPONSE:** We amended the text to reflect this comment

**Replies Reviewers' comments:**

**Reviewer #1:**  
*Manuscript Summary:*  
The authors investigated the properties of nanocellulose produced from bleached eucalyptus pulp (BEP) and an unbleached mixed hardwood kraft pulp (UMHP) fibers using fully recyclable di-carboxylic solid acids. The results obtained are quite interesting and the authors have fully and perfectly addressed the points raised by the previous reviewers. Therefore, I recommend that this manuscript be accepted for the publication in the Journal of Visualized Experiments.  
  
*Major Concerns:*  
N/A  
  
*Minor Concerns:*  
N/A  
  
*Additional Comments to Authors:*  
N/A  
  
  
**Reviewer #2:**  
*Manuscript Summary:*  
The paper by Bian et al. contains a novel method to produce cellulose nanocrystals (CNC) and cellulose nanofibrils (CNF) by a weak acid hydrolysis at elevated temperatures.  
  
*Major Concerns:*  
The produced nanocelluloses are inferior to CNC and CNF produced by other methods, as they have much larger diameters and much lower axis ratios. The authors claim that the main advantages of their method are that it is "green" and produces nanocellulose which is thermally more stable. How more green their method is can be debated, as acids or oxidants used in the production of CNC can be recovered and re-used. It seems to me that CNC and CNF produced by this new method is inferior in many respects, compared to conventional CNC and CNF, because of their small aspect ratios. Hence the uses of this nanocellulose will be limited. The authors should put more effort in reducing the aspect ratio to values comparable with other nanocelluloses, if this method is going to be adapted by others.

**RESPONSE:** Aspect ratio is only one measure of the cellulose nanomaterials. It can be tuned as we demonstrated previously. This paper is a method paper to show people how this method can be implemented.

*Minor Concerns:*  
The authors provide insufficient and conflicting data, regarding the dimensions of the CNC and CNF produced. In the abstract it is mentioned that CNC lengths vary from 239-485 nm, whereas mean lengths of BEC and UMHP are given as 239 and 336 nm (line 219). Surely if the mean is 239 nm, a large fraction is smaller than 239 nm, contradicting the range given in the abstract. Why are no standard deviations given for length and width? For CNF no diameters and length are given at all, just average aspect ratios for the two pulps.

**RESPONSE**: We have corrected in the abstract.

It is claimed that the improved thermal stability is due to improved crystallinity lines (261-262). This is surprising as the crystallinity of conventional CNC is pretty large. The authors should back up their claim with data and/or references to the literature. Has CNF also an improved thermal stability? Surely CNF contains amorphous regions and it would be difficult to ascribe improved thermal stability to crystallinity for CNF.

**RESPONSE:** We disagree with the reviewer on the crystallinity issue. The present authors have done a substantial amount of work on the subject. The current understanding on cellulose crystallinity is still very limited due to incapable of accurately measure cellulose crystallinity. All measurements are ensemble measurement of a cellulose pellets. The reviewer is advised to check our early work (Chen et al., Cellulose 23:1753), CNC crystallinity by sulfuric acid hydrolysis is very low. The reviewer is also advised to check our recent review paper on subject (Chem Rev. DOI: 10.1021/acs.chemrev.6b00225, 2016)

*Additional Comments to Authors:*  
N/A  
  
  
**Reviewer #3:**  
*Manuscript Summary:*  
I do not know JOVE as a journal, so please take any comments with a grain of salt. However, I think the topic of the manuscript is timely, needed and interesting. Scalable methods for highly dispersible acid functionalized CNC and CNF is a topic of current interest. The authors describe a unique and easy way to produce them.  
  
*Major Concerns:*  
More characterization of the materials is needed, although this is probably best suited for a followup paper.

**RESPONSE:** The comment is well taken. Agree that this is a method paper.

*Minor Concerns:*  
The procedure was written in a non-standard method. It came off as a recipe or "directions" as opposed to the more standard "description". I suggest rewriting it to a more standard tone. However, this may be the manner of JOVE, I do not know.

**RESPONSE:** Yes. We have to rewrite the description according to Journal language. We have spent so much time doing that and continuing doing that.

*Additional Comments to Authors:*  
N/A