Your manuscript, JoVE58707 Measuring and understanding large amplitude oscillatory shear: A case study of concentrated polymeric systems, has been editorially and peer reviewed, and the following comments need to be addressed. Note that editorial comments address both requirements for video production and formatting of the article for publication. Please track the changes within the manuscript to identify all of the edits.  
  
After revising and uploading your submission, please also upload a separate rebuttal document that addresses each of the editorial and peer review comments individually. Please submit each figure as a vector image file to ensure high resolution throughout production: (.svg, .eps, .ai). If submitting as a .tif or .psd, please ensure that the image is 1920 pixels x 1080 pixels or 300 dpi.  
  
Your revision is due by **Aug 14, 2018**.  
  
To submit a revision, go to the [JoVE submission site](http://www.editorialmanager.com/jove#_blank) and log in as an author. You will find your submission under the heading "Submission Needing Revision".  
  
Best,  
  
Alisha DSouza, Ph.D.  
Senior Review Editor  
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You will find Editorial comments and Peer-Review comments listed below. Please read this entire email before making edits to your manuscript.  
NOTE: Please include a line-by-line response to each of the editorial and reviewer comments in the form of a letter along with the resubmission.   
  
**Editorial Comments:**  
  
• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

We have proofread the manuscript a couple of times to make sure there are no spelling or grammatical errors.

• Please reduce the number of keywords/phrases to at most 12.

The change has been made.

• Please simplify the title and avoid using the colon in the title if possible.

The title has been simplified to, “Measuring and understanding the large amplitude oscillatory shear response of soft materials”.  
  
• **Introduction:** Please ensure that appropriate references to published literature are provided.

Appropriate references are provided throughout the manuscript.

• **Protocol Language:**The JoVE protocol should be almost entirely composed of numbered short steps (2-3 related actions each) written in the imperative voice/tense (as if you are telling someone how to do the technique, i.e. "Do this", "Measure that" etc.). Any text that cannot be written in the imperative tense may be added as a brief “Note” at the end of the step (please limit notes). Please re-write your ENTIRE protocol section accordingly. Descriptive sections of the protocol (e.g. Lines 143-158) can be moved to Representative Results or Discussion. The JoVE protocol should be a set of instructions rather a report of a study. Any reporting should be moved into the representative results.

We have rewritten the protocol and made the changes required.

• **Protocol Detail:** Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. **Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc) to your protocol steps.** There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

Details have been added to the protocol steps.

• **Protocol Numbering:** Please adjust the numbering of your protocol section to follow JoVE’s instructions for authors, 1. should be followed by 1.1. and then 1.1.1. if necessary and all steps should be lined up at the left margin with no indentations. There must also be a one-line space between each protocol step.  
The changes have been made.

• **Protocol Highlight:** Please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps.

1) The highlighting must include all relevant details that are required to perform the step. For example, if step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be included in the highlighting.  
2) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.

3) Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.

4) Notes cannot be filmed and should be excluded from highlighting.

5) Please bear in mind that software steps without a graphical user interface/calculations/ command line scripting ( e.g. section 3,4 ) cannot be filmed.

6) Please ensure that the manuscript title best reflects the filmable content (i.e. the portions you highlight).

A portion of the protocol to be visualized is highlighted.

• **Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.  
The changes are made according to the comments.

• **Figures:** Please remove the embedded figures from the manuscript. Figure legends, however, should remain within the manuscript text, directly below the Representative Results text.  
We have removed the figures while the captions are placed below results text.

• **Commercial Language:**JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Examples of commercial sounding language in your manuscript are TwinDrive, RheoCompassTM, Anton Paar, Origin or Excel, Matlab, etc.

1) Please use MS Word’s find function (Ctrl+F), to locate and replace all commercial sounding language in your manuscript with generic names that are not company-specific. All commercial products should be sufficiently referenced in the table of materials/reagents. You may use the generic term followed by “(see table of materials)” to draw the readers’ attention to specific commercial names.

The use of commercial names have been removed, with the exception of MATLAB. MATLAB is the key software to implement the theme software in the protocol and the authors think it is important to let users know that it is the platform our software is constructed on. Many articles in JoVE have taken the same approach with a lab-made software, mentioning “MATLAB” by name to inform the readers. (Please see the three examples below) We have greatly reduced the use of the term.

Ibáñez-Ventoso, C., Herrera, C., Chen, E., Motto, D., Driscoll, M. Automated Analysis of *C. elegans* Swim Behavior Using CeleST Software. *J. Vis. Exp.* (118), e54359, doi:10.3791/54359 (2016).

Nikolaus, J., Karatekin, E. SNARE-mediated Fusion of Single Proteoliposomes with Tethered Supported Bilayers in a Microfluidic Flow Cell Monitored by Polarized TIRF Microscopy. *J. Vis. Exp.* (114), e54349, doi:10.3791/54349 (2016).

Rajagopal, V., Bass, G., Ghosh, S., Hunt, H., Walker, C., Hanssen, E., Crampin, E., Soeller, C. Creating a Structurally Realistic Finite Element Geometric Model of a Cardiomyocyte to Study the Role of Cellular Architecture in Cardiomyocyte Systems Biology. *J. Vis. Exp.* (134), e56817, doi:10.3791/56817 (2018).

• Please define all abbreviations at first use.

We have made sure the abbreviations are defined at first use.

• Please use standard abbreviations and symbols for SI Units such as µL, mL, L, etc., and abbreviations for non-SI units such as h, min, s for time units. Please use a single space between the numerical value and unit.

All the changes are made according to the comment.

• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."  
The results here are all new and have not been reported elsewhere.

**Comments from Peer-Reviewers:**

We thank all reviewers for their detailed comments and questions. We believe that addressing the comments and questions in the manuscript have strengthened it greatly.

**Reviewer #1:**

The paper describe a procedure to analyze and interpret the LAOS experiments using a Matlab code, based on the Sequence of Physical Processes (SPP) technique. The shown procedure is useful for experimentalists, but brings no novelty for the topic. The results and interpretation are more or less classic, but the work is useful for newcomers in the field.

I have two recommendations/observations for the authors:

1. To point very clear if the tests are strain or stress controlled. Ideal should be to perform both and to analyze the differences .... (maybe in a future work!).

The rheometer used in this study can be configured into either a stress-controlled or a strain-controlled instrument. As strain-controlled LAOS is desired in this study, we have configured the rheometer into a typical strain-controlled rheometer, where the upper motor operates solely as a torque transducer and the bottom motor acts as a drive unit. We have made the point stronger by including a note in the instrument setup section that a typical strain-controlled configuration is used in the study.

We have also replicated the same measurements on another strain-controlled rheometer (an ARES-G2 from TA Instruments) to confirm that the responses are consistent.

We agree that it would be interesting to perform some both strain- and stress-controlled experiments in the future and analyze the differences.

2. To show the evolution of the Lissajous Figures with the strain amplitude sweep curve (corresponding between Fig. 4a and Fig. 4b with Fig. 4d, see the paper from Polymer 104 (2016) 215-226).

We thank the reviewer for the comment. The suggested paper in Polymer is interesting and we enjoyed reading it. We also like the style the authors used to correlate the figures. Nonetheless, the figures in the current manuscript, shown as Figs 4a, 4b, and 4d, have a clear correspondence because of the strain amplitudes that are easily read off the abscissa. We have also used different colors across Figs 4a and 4b to more clearly differentiate between separate curves. We therefore do not think that extra information on the amplitude sweep plot is required. We aim to use the amplitude sweep (Fig. 4d) to emphasize the correspondence between maximum across amplitudes and the value of linear regime G’.

I think the format and the work is suitable for this type of publications. Therefore, if the authors take into account my observations, I recommend the paper to be published in Journal of Visualized Experiments.

**Reviewer #2:**

Manuscript Summary:

This paper tries to give explanation about SPP analysis under LAOS flow for two polymer solution. It is very interesting. Especially, visualization of LAOS test is very meaningful. Therefore, I think publication of this paper should be considered. However, I listed some my questions (a few minor questions).

Major Concerns:

No

Minor Concerns:

Page 3, line 123-124

The authors said "two concentrated polymer suspension".

But, in my opinion, XG solution and PEO solution is not suspension but polymer solution.  
Suspension can make confusion to general reader.

This appear at other page of manuscript. I suggest to change it.

That is an excellent point and we agree that they are in solution form, not suspension. We did change all the wording in the manuscript accordingly.

**Reviewer #3:**

Manuscript Summary:

This manuscript reports the use of matlab analysis feature along with experimental measurements on a commercial rheometer. Given that the large deformation behaviour of soft matter is of great interest, several methods have been proposed, and the protocol presented in this work adds to this. However, I have some concerns regarding the manuscript, especially since it is intended to be a "method" report.  
  
Major Concerns:  
1. With a new method, it would be helpful to show some idealized response. Generally, we might want to talk about Newtonian fluid, crosslinked rubber, Maxwell fluid (material with a single relaxation time) etc, while introducing viscoelastic response. Are there no such idealized responses which can be shown here? This manuscript reports results with two polymeric solutions, and given their complex response, I am afraid that efficacy of method is not well brought out.

We do not intend for the current manuscript to report a brand new method of analysis. Instead, it aims to instruct readers as of how to correctly measure and understand a LAOS response with a published framework that has already been used in a number of studies. For the exemplary use of the method, please see *Korea-Australia Rheology Journal* **29** (4), 269–279. 2017 and *Journal of Rheology* **62** (4), 869–888. 2018

We agree that some simple examples should be shown when proposing a new analyzing framework. In fact, when the framework was first proposed (*Rheologica Acta* **56** (5), 501–525. 2017), results from several models were shown, including generalized Newtonian fluid (p.510), the elastic Bingham model (p.511), and the corotational Maxwell model (P.512). Experimental results from a Maxwellian material with a single relaxation time are also shown in Figs. 6-8 to inform readers as how these rheologically-simple models/materials behave.

The current manuscript, by contrast, aims to apply the technique to somewhat more complex materials such as the investigated polymer solutions, and guide the readers through the process of understanding the complex response from these materials.

2. Figures 3 and 4(c)/5(c) are backbones for the discussion of analysis based on the proposed protocol. However, 4(c)/5(c) have not been given adequate attention during discussion. For example, the manuscript states that "To fully understand the intra-cycle physics, the time-dependent Cole-Cole plots obtained from the SPP freeware are presented in Figures 4(c) and 5(c). Interpretations of the plots are discussed in the manner laid out by the legend in Figure 3." However, I did not notice the interpretations as claimed in this sentence.

We have addressed this and made the point stronger. Please see the following revised sentences.

“To fully understand the intra-cycle physics, the time-dependent Cole-Cole plots obtained from the SPP freeware are presented in Figures 4(c) and 5(c). Interpretations of the plots are discussed in the manner laid out by the legend in Figure 3, where the relative motion of trace quantitatively indicates whether the materials undergo stiffening/softening or thickening/thinning in an intra-cycle sense.”

Also, a more detailed interpretation is specifically provided in Fig. 7, where we break a single oscillation into four distinct processes, to guide readers to better understand the physical processes in an oscillation.

3. As a method reporting, would it not be useful to report other measures (mentioned in the manuscript) and demonstrate how the current protocol is "better" or "complementary"?

Other commonly-used methods include Fourier-transform rheology and the Chebyshev description, which have been shown to be trivially related. Fourier transform-based frameworks analyze the nonlinearity of stress response and report corresponding parameters, including I3/I1, Q and Q0 etc. These parameters are useful in terms of mathematically quantifying the distortion of stress, but clear physical interpretations that might allow an experimentalist to connect them to the physical process a material undergoes is still missing from the literature. This lack of physical meaning somewhat restricts experimentalists from further applying these methods and making correlations with structural parameters. For instance, the structural information obtained from either simulation or scattering techniques are typically time-resolved, yet FT-based frameworks all average the stress response over a complete cycle of deformation.

One of the advantages of the SPP framework is that the local information regarding elastic and viscous components can be universally defined, and the physical meanings of these parameters are clear. This local information is useful and valuable to scientists interested in the correlation between bulk rheology and microstructure evolution. Please see J. Rheol. 62. 2018 p.869-888.

We have pointed out the limitations of these other methods in the introduction and provided specifics regarding how SPP provides a better understanding than the other techniques.

Minor Concerns:

Definitions of transient, differential, steady viscosity - please check the consistency/correctness in y-axes labels / caption in Figure 6 and the adjoining discussion in lines 388-391.

We have made changes in the manuscript accordingly. The viscosity determined from is now referred as “transient differential viscosity”, and the one determined from a steady-shear test is called “steady-shear flow viscosity.” The wordings are used consistently throughout the manuscript.

**Reviewer #4:**

Manuscript Summary:

A useful method for analyzing LAOS data within the SPP method is presented and illustrated for two typical polymeric fluids. The presentation is concise and informative and the method should be fairly easy to follow (although I do not have access to the software to determine if indeed it functions as suggested). The manuscript is well written and the major concerns can probably be easily handled in revision.  
  
Major Concerns:

\* As the method is software based, and although it is listed as available upon request, should it not be part of the JOVE publication?

This is a really good suggestion, and we appreciate the reviewer’s point. We have now attached the software package to the article so that it is available for readers to download as a supplementary file.

\* Two polymer solutions are tested and presented- but not clearly compared and contrasted. The strength of the method lies in part in being able to distinguish between classes of material behavior. It is strongly suggested to add one paragraph comparing and contrasting the results of the method for the two polymers. In short, what can we learn?

Excellent point. A paragraph comparing the results from the gel-like XG and the concentrated entangled PEO solutions has been added to address the comment. Please see lines #450-465.

\* Temperature control is very, very important in rheology and it was not clear how this was achieved and to what level of accuracy?

The method to control temperature is added in the protocol part.

In the current study the temperature is controlled at 25±0.1 °C and 35±0.1 °C for XG and PEO solutions, respectively, which is included in a note in the protocol section. (line #184-186)

\* Slip is always a problem when going into a nonlinear measurement. in the FT method of Wilhelm, slip can be detected - can this be detected in the SPP method?

We would argue slip is not necessarily always a problem in nonlinear measurements. There are soft materials that have been confirmed not to slip under shear with direct-imaging techniques. Please see as an example *Phys Rev Lett*, 98(19), p.8357, 2007. Other studies have also shown that with roughened geometries slip can be prevented.

We have performed the same flow conditions with a roughened geometry to make sure that slip is not an issue in the investigated materials. Good agreement is seen with different geometries and a no-slip assumption is therefore supported. A number of studies investigating the same materials did not report slip issues with smooth geometries as well. As an example, please see *J. Non-Newtonian Fluid Mech.* 107 (2002) p.51-65 or *Fibers and Polymers.* 7. (2) 2006 p.129-138 for the study of xanthan gum solutions, and please see *Polymer* 104. 2016 p.171-178 and *Rheol Acta* 52. 2013 p.841-857 for the study of PEO solutions.

A few studies reported that FT-rheology is capable of interpreting slip. For instance, one work (*Macromolecules* 40. 2007 p.4250-4259) proposed to use a sinusoidal, a rectangular, a triangular, and a saw tooth wave as a basis and the time domain signal is described by these characteristic functions to interpret linear behavior, softening, stiffening, and slip or shear bands. Because these four characteristic waves are not orthogonal to each other, the interpretation from the idea of this superposition cannot be generally applied. That is, the results that one obtains from the fitting will be determined by the order in which one fits each characteristic function. Slip, in this case, is therefore not clearly interpreted.

Another common approach in FT-rheology to interpret slip from the presence of even harmonics. However, even harmonics represent an asymmetric stress response and slip is only one of many possible causes of the observable even harmonics. A transient unsteady-state response, shear banding, or a thixotropic behavior could also give rise to even harmonics. Further, if slip happens in a symmetric manner, then even harmonics would also fail to capture the behavior, and the slip would be conflated with the odd harmonic response. We think the information from Fourier transform rheology is useful, but cannot “detect” slip without complementary imaging techniques for the above reasons.

The SPP technique does not contain any information that is not contained in the FT-based approaches. The argument that has been made in favor of the SPP approach is one of presentation: the SPP approach has been shown to present the data contained in LAOS experiments in such a way as to clearly relate back to the physics of the materials or models under deformation.

Minor Concerns:

\* The abstract needs to concisely explain the value of the contribution. It can also have less of "we..." - who else?

The reviewer’s point is well made. We have rewritten the abstract to strengthen the value of the contribution, with several major points of the study added. The sentences starting with “we…” have been revised as well.

\* P3 LAOS may not be that representative of the nonlinear flows typical in processing because these seldom involve flow reversals such as observed in LAOS. The comments about this should be qualified.

Excellent point. The following sentence is included in the manuscript to make the distinction that we use LAOS to approximate the practical conditions that may not necessarily involve flow reversal.

“Although these practical conditions may not necessarily involve flow reversal as in oscillatory shear, the flow field can be easily approximated and tuned with the independent control of applied amplitude and imposed frequency in an oscillatory test. Furthermore, the SPP scheme can be used as described here to understand a broad range of flow types, including those that do not include flow reversals such as the recently-proposed UD-LAOS22, in which large amplitude oscillations are applied in one direction only (leading to the moniker “uni-directional LAOS”). For simplicity, and for illustrative purposes, we restrict the current study to traditional LAOS, which does include periodic flow reversal.”

\* P7 A stress controlled rheometer is used to do strain controlled LAOS. Some proof of the accuracy of this must be shown.

The rheometer used in this study can be configured as either a stress-controlled or a strain-controlled instrument. As strain-controlled LAOS is desired in this study, we have configured the rheometer as a typical strain-controlled rheometer, where the upper motor operates solely as a torque transducer and the bottom motor acts as a drive unit. We have made the point stronger by including a note in the instrument setup section that a traditional strain-controlled rheometer is used in the study.

“Note: The MCR-702 rheometer can be configured in either a CMT (combined motor-transducer) or SMT (separate motor transducer) modes. With only a single motor integrated in the rheometer head, it acts as a traditional CMT stress-controlled rheometer and the data obtained require inertia corrections. With two motors incorporated in a SMT mode, the upper motor operates solely as a torque transducer and the bottom motor acts as a drive unit thus converting the rheometer into a strain-controlled rheometer.”

We have also replicated the same measurements on another strain-controlled rheometer (an ARES-G2 from TA Instruments) to confirm that the responses are consistent.