August 2nd, 2016

Attn: Jaydev Upponi, Benjamin Werth

Science Editor – Chemistry

Journal of Visualized Experiments

One Alewife Center

Suite 200

Cambridge, MA 02140

USA

**Object: Post-peer-review Revision of Manuscript to the Journal of Visualized Experiments**

Dear Jaydev Upponi,

I would like to thank the reviewers for their comments on my manuscript "In Situ Visualization of the Phase Behavior of Oil Samples under Refinery Process Conditions". Please find in the next pages a detailed response to the topics discussed by the reviewers.

Best regards,

Cedric Laborde-Boutet

**Editorial comments:**

•Formatting:  
-Please include spaces between all steps and substeps.  
-Please include an acknowledgements section.  
-References – Please include DOI where available.

These formatting issues have been addressed in the revised version of the manuscript.

•Grammar:  
-Please remove instances of “Please” from the manuscript. This is not appropriate for the protocol.  
-2.1.2, 5.1.1 – Please use imperative tense or convert to a note.

These issues have been addressed in the revised version of the manuscript.

•Visualization: Please include photographic images of the setup (micro-reactor and microscope) to aid in visualization. These should be submitted as supplemental files.  
  
•Additional detail is required: 1.10 – How is this checked?

This detail is described in the Note which follows 1.10.

•Branding: 2.4 – Jubilee

The term “jubilee clip” was replaced by “hose clip” in the revised version of the manuscript.

**Reviewers' comments:**  
**Reviewer #1:**  
*Manuscript Summary:*  
The manuscript "In Situ Visualization of the Phase Behavior of Oil Samples under Refinery Process Conditions" provides interesting and potentially useful information in a high temperature microscopy apparatus. However, the manuscript should be improved to meet the criteria of a good scientific paper.  
-In general terms, the manuscript lacks from a literature review in the focus of the article that is the apparatus, focusing instead on the application of similar apparatus in the literature. It would be interesting if the review were made based on apparatus differences and not only applications.

A comparison of the present experimental device with alternative methods is presented in the Discussion, under the “Significance of the technique with respect to existing/alternative methods” sub-section. More specifically, this section describes the performance of alternative designs, such as top-down configuration of the usage of other types of windows.

- It also shows some theoretical discussions and calculations without describing how they were performed, it would be useful if the calculations were fully disclosed.

Calculations of the Hue, Saturation and Intensity are presented in 5.1. The captions pertaining to the figures which present color or brightness data were slightly modified accordingly.

-The manuscript describe in detail the experimental procedure, but fails to describe the apparatus, only figure captions are presented. The apparatus must be described in detail.

The apparatus is described in a supplemental file. Pictures were added to help with the description of the setup components.

-The list of figure captions is included in the middle of the manuscript. It should be included in the end and each Figure must be discussed in the text, besides the Figure labels (A, B, C, D) must be used to refer to a given Figure. In the present form, the manuscript reminds us a lab manual. Please discuss the results.

The list of figure captions was placed as advised by the Instructions for Authors document from JoVE. The present manuscript aims to describe the experimental method, with an overview of the relationships between image properties and the physico-chemical characteristics of the samples. Further discussion about the visual evolution of reacting materials is beyond the scope of the work for publication in JoVE.

-Line 29: "…visualization of opaque samples such as petroleum vacuum residues or asphaltenes." Other compounds may be visualized by reflected polarized light, like waxes, if the crude oil has them.

The present technique is not limited to the visualization of anisotropic material (such as waxes).

-Line 31: "…of the microscope on the light path allows high-contrast imaging of isotropic and anisotropic media." Is impossible "to see" isotropic materials in polarized light. I suggest rewrite for some more clearly explanation. Maybe use "versus" between iso and anisotropic. The isotropic media is the dark part of the image, in theory you "do not see".  
-Line 68: "This system is unique in that it allows for the visualization of opaque isotropic material…" I suggest to insert a brief explanation of how this is possible in this system.

The ability for visualizing both isotropic and anisotropic media is the main subject of the Discussion, under the “Significance of the technique with respect to existing/alternative methods” sub-section.

-Line 135: No image is shown to support the claim.

This protocol step is actually rather trivial; additional pictures were added to the setup description in the supplemental file to clarify it. The video should make it self-explanatory.

-Line 245: "Note: For future quantitative image analyses, snapshots should be taken with the same magnification (preferably 100 x), lighting conditions (preferably maximum lighting), and camera acquisition settings (linear photosensitivity response, with the appropriate exposure time to balance image brightness - typically between 200 ms and 400 ms for heavy oil samples)." These conditions are good for this system and samples. However for other samples maybe this parameters need change. Thus, do not impose the parameters, for example 100x magnification, or 200 an 400 ms. Write that information like a suggestion for an specific Athabasca Vacuum Residue sample.

This paragraph was reworded more appropriately in the revised version of the manuscript to describe that microcraphs should be taken with consistent settings throughout an experiment. Additionally, the list of settings is given as a guideline.

-Line 354: "The formation of mesophase has the effect of increasing the overall brightness intensity and enhancing the blue color shift." Add reference.

A reference was added in the revised version of the manuscript.

-Subtitle: Figure 4. "Micrographs taken during a thermal cracking experiment on Athabasca…" Improve the subtitle. What type of micrographs? Polarized? Is the first one polarized light? The background is not dark as expected.

All pictures were taken under cross-polarized light unless noted. Indeed, the background is not dark, which shows the ability for the setup to image isotropic media, as explained in the Discussion, under the “Significance of the technique with respect to existing/alternative methods” sub-section.

-Paragraph: "Color changes follow the evolution of the spectral properties of the sample which correspond to its chemistry. Most notably, vacuum residue samples have exhibited a red-to-blue color shift when subjected to thermal cracking reactions for an extended amount of time, yet prior to the formation of sediments. Given enough thermal cracking reaction time, such samples undergo an increase in aromaticity and begin to form oligomers. The formation of more conjugated species leads to a change in spectral properties, where the predominant light absorption of the sample shifts from shorter wavelengths to longer wavelengths. Since reflection spectra are the counterpart of absorption spectra, the corresponding spectral shift in the reflected light goes from longer wavelengths to shorter wavelengths, matching the color change from red to blue." Add some references.

A reference was added in the revised version of the manuscript.

-Line 509: "Conversely, hydrocoversion…. " No image is shown to support the claim.

A figure was added to illustrate this observation.

-Figure 1: Improve this figure. The focus is on the reactor, but here the microscope is in evidence. It is expected that everyone will read the paper know what an inverted microscope is. Thus, only draw a small part of the microscope.

Figure 1 was relocated as Figure S 1 (supplemental file). This figure was revised to zoom in the section pertaining to the reactor inside the heater, as recommended by this comment.

*Major Concerns:*  
N/A  
  
*Minor Concerns:*  
N/A  
  
*Additional Comments to Authors:*  
N/A  
  
  
**Reviewer #2:**  
*Manuscript Summary:*  
The manuscript describes a new instrument that can be used to follow visually chemical reactions likely to happen in a refinery. The methodology is described in great detailed and the complications that might arise from the use of the instrument during the tests are clearly explained. This technique can have applications in other areas of the petroleum business such as in the study of phase separations (asphaltene and waxes) as well as in demulsification processes in upstream and downstream operations. The manuscript should be published with some minor changes. In particular, other potential applications of this technique should be added to the manuscript.

The sub-section “Future applications or directions after mastering this technique” was expanded to include other potential applications.

*Major Concerns:*

N/A  
  
*Minor Concerns:*

The cleaning of the reactor after the experiments might be more difficult than what it is mentioned in the text. For regular reactors, the cleaning might require sand blasting when there is coke formation. In the case of the microreactor, the authors indicated the use of a emery cloth. I wonder how effective this is.

The micro-reactor cannot undergo sandblasting since it would very likely compromise the integrity of the sealing surfaces. Coarse grit emery cloth was found suitable to removing the layers of coke – the “metallic shine” is apparent at the end of the cleaning procedure. A note pertaining to the reactor cleanup was expanded to describe this.

Also, in those situations where hard coke is formed, how easier would be to avoid damage to the sapphire window during cleaning.

In our experience, coke does not adhere much to the sapphire surface and can be removed with solvent-soaked cotton swabs with relative ease. It is much easier to remove coke from the sapphire surface than it is to clean the coked stainless steel surfaces.

*Additional Comments to Authors:*

N/A  
  
  
**Reviewer #3:**

*Manuscript Summary:*

Well written manuscript

*Major Concerns:*

Although the SOP is well written, I am concern for the new scientists entering to this field, they may have hard time to follow the protocol. Better picture of the breakdown of the equipment (different parts) may help to understand the system better.

Figure S 1 (formerly Figure 1) was revised to zoom in the section pertaining to the reactor inside the heater. In addition, Figure 1 was added to provide a better description of the micro-reactor.

*Minor Concerns:*

Better and more clear microscopic pictures.

The Pictures presented on Figure 3 and Figure 11 are among the best that this technique can provide. Pictures presented on Figure 9 and Figure 10 were purposefully shown to illustrate the lack of visual evolution if an isotropic window material is used, or if bright-field (unpolarized) lighting is used.

*Additional Comments to Authors:*

N/A