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In Situ Visualization of the Phase Behavior of Oil Samples under Refinery Process Conditions

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Abstract:	<p>To help addressing production issues in refineries due to the fouling of process units and lines, we have developed a setup as well as a method to visualize the behavior of petroleum samples under process conditions. The experimental setup relies on a custom-built micro-reactor fitted with a sapphire window at the bottom, which is placed over the objective of an inverted microscope equipped with a cross-polarizer module. Using reflection microscopy enables the visualization of opaque samples such as petroleum vacuum residues or asphaltenes. The combination of the sapphire window from the micro-reactor with the cross-polarizers module of the microscope on the light path allows high-contrast imaging of isotropic and anisotropic media. While observations are carried out, the micro-reactor can be heated to the temperature range of cracking reactions (up to 450 °C), subjected to H₂ pressure relevant to hydroconversion reactions (up to 16 MPa) and can provide stirring to the sample by magnetic coupling.</p> <p>Observations are typically carried out by taking snapshots of the sample under cross-polarized light at regular time intervals. Image analyses may not only provide information on the temperature, pressure and reactive conditions yielding phase separation, but can also give an estimate of the evolution of chemical (absorption/reflection spectra) and physical (refractive index) properties of the sample before the onset of phase separation.</p>
Author Comments:	<p>The supplied figures are relevant to several sections of the manuscript (Protocol steps, Representative results, Discussion). However, figure captions were all grouped in the "Representative Results" section. I have inserted bracketed comments regarding where to place the figures which do not correspond to the "Representative Results" section.</p> <p>Supplemental files (Document on the Setup Description, Figure S 1 and Figure S 2)</p>

	are supplied additionally to facilitate the understanding of the apparatus usage and protocol steps.
Additional Information:	
Question	Response
If this article needs to be "in-press" by a certain date, please indicate the date below and explain in your cover letter.	



July 18th, 2016
Benjamin Werth
Science Editor – Chemistry
Journal of Visualized Experiments
One Alewife Center
Suite 200
Cambridge, MA 02140
USA

Object: Submission of Manuscript to the Journal of Visualized Experiments

Dear Benjamin Werth,

The attached manuscript, entitled “*In Situ* Visualization of the Phase Behavior of Oil Samples under Refinery Process Conditions”, by Cedric Laborde-Boutet and William C. McCaffrey, is submitted for publication in the Journal of Visualized Experiments. In this article, we present a unique setup and method for the *in situ* visualization of oil samples under reaction conditions pertaining to refining and upgrading processes. Additionally, since the data we obtain is in the form of a series of micrographs, the method lends itself very well to a presentation in video format. As such, the Journal of Visualized Experiments provides an ideal platform for displaying our research.

Please find a list of 6 peer reviewers and their respective affiliation below:

- Kevin J. Smith, University of British Columbia, Department of Chemical and Biological Engineering (kjs@chbe.ubc.ca)
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Yours sincerely,

Cedric Laborde-Boutet

TITLE:

***In Situ* Visualization of the Phase Behavior of Oil Samples Under Refinery Process Conditions**

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KEYWORDS:

Petroleum fouling; *in situ* visualization; oil refining and upgrading; cross-polarized microscopy

SHORT ABSTRACT:

This article describes a setup and method for the *in situ* visualization of oil samples under a variety of temperature and pressure conditions that aim to emulate refining and upgrading processes. It is primarily used for studying isotropic and anisotropic media involved in the fouling behavior of petroleum feeds.

LONG ABSTRACT:

To help address production issues in refineries caused by the fouling of process units and lines, we have developed a setup as well as a method to visualize the behavior of petroleum samples under process conditions. The experimental setup relies on a custom-built micro-reactor fitted with a sapphire window at the bottom, which is placed over the objective of an inverted microscope equipped with a cross-polarizer module. Using reflection microscopy enables the visualization of opaque samples, such as petroleum vacuum residues, or asphaltenes. The combination of the sapphire window from the micro-reactor with the cross-polarizer module of the microscope on the light path allows high-contrast imaging of isotropic and anisotropic media. While observations are carried out, the micro-reactor can be heated to the temperature range of cracking reactions (up to 450 °C), can be subjected to H₂ pressure relevant to hydroconversion reactions (up to 16 MPa), and can stir the sample by magnetic coupling.

Observations are typically carried out by taking snapshots of the sample under cross-polarized light at regular time intervals. Image analyses may not only provide information on the temperature, pressure, and reactive conditions yielding phase separation, but may also give an estimate of the evolution of the chemical (absorption/reflection spectra) and physical (refractive index) properties of the sample before the onset of phase separation.

INTRODUCTION:

The study of the phase behavior of oil samples in a wide range of temperatures, pressures, and reactive conditions can yield very useful information for the operator of a refinery that

processes a variety of feeds. In particular, the fouling of process units and lines by an uncontrolled formation of coke or sediments can severely affect production (loss of throughput) and energy efficiency (increase in heat transfer resistance)¹⁻³. Possible plugging caused by the accumulation of fouling material may require a shutdown for clean-up purposes, which would have a highly negative economic impact⁴. Conducting an assessment of the fouling propensities of feeds can be highly valuable for the optimization of process conditions⁵ and the blending of refinery streams.

We have developed an *in situ* analyzer of petroleum stability in our laboratory to allow the visualization of oil samples subject to refinery process conditions. This apparatus relies on a specifically designed reactor made of stainless steel fittings and equipped with a sealed sapphire window at the bottom. The main principle of the device is the illumination of the sample inside the reactor at the desired range of temperature and pressure and the imaging of the resulting cross-polarized reflection. While previous published work relative to this setup focused on thermal cracking processes to emulate visbreaking conditions⁶⁻⁹ (which do not require high pressure), the reactor design was overhauled to investigate the behavior of samples under hydroconversion (catalytic cracking under high H₂ pressure) and aquathermal¹⁰ (thermal cracking under high-pressure steam) conditions. Thus, the device was revised in order to operate in the 20-450 °C temperature range and the 0.1-16 MPa pressure range, with the ability to sustain both 450 °C and 16 MPa for reaction times of up to 6 h.

The first level of analysis on the visual information of the samples under a particular range of temperature, pressure, and reactive conditions is to determine whether the sample is single-phase or multiphase. This system is unique in that it allows for the visualization of opaque isotropic material and is not limited to the visualization of anisotropic material described in other work¹¹. While the main indicator of the fouling propensity of samples is the tendency to drop sediments out of the bulk liquid; gas-liquid, liquid-liquid, liquid-solid, and more complex phase behaviors can be observed. However, valuable information can also be extracted from the visual evolution of a liquid as it remains homogeneous (single-phase). In particular, the brightness of the images is related to the refractive index and the extinction coefficient of the sample, while the color of the sample is a subset of its spectral information in the visible light range (380-700 nm), which can be used as a descriptor of its chemistry⁹.

PROTOCOL:

Caution: Use all appropriate safety practices when performing an experiment under high temperature and pressure conditions, including the use of engineering controls (H₂ flow limiter, pressure regulators, and rupture disc assembly) and personal protective equipment (safety glasses, temperature-resistant gloves, lab coat, full-length pants, and closed-toe shoes). Consult all relevant material safety data sheets (MSDS) before use. Carry out micro-reactor loading and clean-up in a fume hood, as these steps involve the use of harmful volatile organic solvents (toluene and dichloromethane).

Note: Setup description (see supplemental file).

1. Micro-reactor loading

1.1. Clamp the micro-reactor vertically and upside-down, with the bottom face-seal (thus positioned at the top) open.

Note: At this stage, the sapphire window, the custom-machined magnet, the 1/16" ferrule, the brass pad, and the bottom nut should not be assembled yet.

1.1.1. Ensure that the fittings that are used to connect the micro-reactor to the gas lines are closed.

1.2. Load around 0.6 g of sample into the reactor through the open face-seal using a thin spatula.

1.2.1. If the sample is originally kept in a large container, make a sub-sample before loading the micro-reactor.

1.2.2. To estimate the amount of sample loaded inside the reactor, weigh the container and the spatula before and after loading, and calculate the mass difference.

1.3. Slide the custom-machined magnet onto the thermocouple.

1.4. Slide the 1/16" front ferrule so that the larger circle is facing up.

1.5. Ensure that the sealing surface (*i.e.*, the fitting groove where the sealing ring sits) of the bottom face-seal fitting is clean and dry.

Note: Given the highly viscous nature of most heavy oil samples, it is very likely that the sealing surface got accidentally smeared by the sample during the loading process.

1.5.1. Dip the tip of a cotton swab in toluene and apply it to the sealing surfaces to clean them. Be careful not to drip toluene inside the reactor cavity, which would contaminate the sample.

1.5.2. If cleaning with toluene is required, make sure the sealing surfaces are dry before proceeding to the next step.

1.6. Ensure that the sapphire window is clean and dry.

1.6.1. If the sapphire window is dirty, use a cotton swab soaked in a suitable solvent, and then perform a final wash using acetone to clean the window surfaces; let it air dry.

1.7. Place the sealing ring on the sealing surface, then the sapphire window on the top of the sealing ring, and then the brass pad on the top of the sapphire window; it is preferable to apply tiny, pinhead-sized drops of lubricant on the brass pad.

1.8. Thread the bottom nut on the bottom face-seal fitting while encapsulating the brass pad and the sapphire window. Adjust the bottom nut until it reaches finger-tight position.

1.9. While holding the reactor upside-down, transfer it to a vice. Use a wrench to tighten the bottom nut by 90° from finger-tight position.

Note: After this step, the reactor does not need to be held upside-down any longer.

1.10. Check the micro-reactor for potential defects in the seal.

Note: The window may show some chips or cracks, or a faulty seal can be identified if the compressed surface of the seal on the window does not make a continuous circle.

1.10.1. In case of a defect, open the micro-reactor for inspection.

1.10.2. After taking remedying action, use a brand-new sealing ring when attempting to reseal the reactor.

2. Micro-reactor installation

2.1. Once the micro-reactor is loaded and sealed, connect the reactor to the gas lines and perform tests for leaks.

2.1.1. Always begin leak testing by using N₂ at a maximum pressure of 5 MPa.

Note: The preferred method for leak testing is the pressure-decay test, where the setup is pressurized and then isolated from the cylinder (closing valves V2 and V3). If the pressure remains stable for an extended amount of time (more than 30 min), no leak is observed.

2.1.2. Carry out additional leak tests at higher pressures if the target pressure for the upcoming experiment is higher than 5 MPa.

Note: These additional leak tests can be performed with maximum pressure increments of 6 MPa until the desired pressure condition for the experiment is matched. Consider 16 MPa as the upper limit of pressure for both leak testing and setup operation.

Note: If the gas used to pressurize the setup in the upcoming experiment is not inert (such as flammable gases), carry out another series of leak tests using the target gas contingent on a successful series of leak tests with N₂.

2.2. After successful leak tests, depressurize the setup before undertaking the next installation steps.

2.3. Place the micro-reactor in the stainless steel heating block, which is itself inserted in the coil heater. Position the assembly on the platform located above the microscope objective.

2.4. Encase the reactor, the heater, and the heating block with the two halves of a casing filled with ceramic wool. Clamp the two halves of the casing together using a hose clip.

2.5. Fine-tune the position of the reactor over the microscope objective.

2.5.1. Turn the microscope on using cross-polarized light. Adjust the vertical position of the objective using the lowest magnification so as to focus on the inside surface of the sapphire window.

2.5.2. Position the reactor so that the field of view at the lowest magnification (typically 50x) covers a radial portion of the window surface where the inner boundary comprises the edge of the 1/16" front ferrule, as described in Figure 1. Photographs of the micro-reactor, held upside-down by a clamp. Pre-loading arrangement, with the bottom face opened (A). The loaded and sealed micro-reactor (B).

2.5.3. **Figure 2**

Note: Actual micrographs acquired by the software should be centered subsets of this field of view, which would avoid showing the ferrule itself.

2.6. Connect the thermocouple of the micro-reactor (TT1) to the temperature controller (TIC1).

2.7. Turn on the motor driving the external magnet to a speed of 120 rpm.

2.8. Pressurize the setup to the desired set-point.

Note: Atmospheric pressure runs are performed by opening all outlet valves to the vent. Batch experiments can be performed by closing valve V4. Experiments under a constant head of pressure (preferable for high-pressure conditions) can be carried out by using the back-pressure regulator PV2.

3. Regular procedure for the visualization of cracking reactions

3.1. Throughout the entire experiment, place the microscope objective under the reactor only when visualizing the sample or taking a snapshot. Avoid leaving the microscope objective under the reactor when it is not needed.

Note: Leaving the microscope objective under the reactor at high temperatures will cause an artificial brightening of the images, resulting in poor data, and may lead to a deterioration of the objective.

3.2. Turn the temperature controller on and apply a temperature set-point of 200 °C. Once the sample temperature reaches 200 °C, perform a round of verifications.

Note: A round of verifications entails verifying the pressure, temperature, reactor position, focal distance of the microscope objectives, and stirring. As the temperature changes, the platform supporting the reactor and the heating assembly deforms slightly, so the vertical position of the microscope objective must be adjusted for the sapphire/sample interface to remain in focus. Stirring can be detected by the motion of the 1/16" ferrule or of small heterogeneity in the sample (such as small mineral solids).

3.3. If everything is in order as the sample reaches 200 °C, perform a set-point change to 300 °C. Once the sample temperature reaches 300 °C, perform another round of verifications.

3.4. Repeat the previous step, with 350 °C as the new temperature set-point.

Note: 350 °C can generally be considered as the higher temperature limit where cracking reactions are not significant (in the time scale of min).

3.5. Change the set-point temperature to the desired reaction temperature, generally in the 400-450 °C range.

3.6. After making the final temperature set-point change, begin monitoring the reaction and recording data at regular time intervals, preferably every min.

3.6.1. Carry out each step of data recording as follows: rotate the nosepiece of the microscope to place the objective under the reactor. Adjust the focus. Take a snapshot. Rotate the nosepiece to move the objective away from underneath the reactor. Note the temperature.

Note: For future quantitative image analyses, snapshots should be taken with consistent settings throughout the experiment, namely in terms of magnification, lighting conditions, and camera acquisition settings (photosensitivity response and exposure time). As guidelines, the micrographs presented in this manuscript were taken with 100x magnification, maximum lighting conditions (using a halogen lightbulb), linear sensitivity response of the camera, and exposure times ranging from 200-400 ms.

3.6.2. Perform the data recording steps repeatedly for as long as needed.

Note: Generally, the duration of the observation is guided by visual changes in the sample (color, brightness, and heterogeneity) or by an estimate of reaction conversion.

Note: Preferably, avoid continuing the experiment after the formation of large amounts of mesophase coke (which makes the reactor more difficult to clean).

4. Shutdown and clean-up

4.1. Terminate the experiment by turning the temperature controller and the stirrer off and depressurizing the setup. Let the reactor cool.

Note: Cooling the reactor can be facilitated by removing the micro-reactor from the casing and out of the heating assembly. Applying a cool air flow to the micro-reactor can also make this process faster and easier.

4.1.1. Once the micro-reactor is cooled to room temperature, disconnect it from the gas lines of the setup, place it in a vice to loosen the bottom nut, and unseal the micro-reactor.

4.2. In a fume hood, take the micro-reactor apart by removing the bottom nut, the brass pad, the sapphire window, the 1/16" ferrule, and the magnet. Remove the sealing ring.

Note: Coke may have formed during the experiment, which may cause the 1/16" ferrule and the magnet to be stuck to the thermocouple.

4.2.1. Use tweezers to pull the 1/16" ferrule and the magnets out. Use a spatula to lever the sealing ring out of the sealing groove. However, take care not to scratch the sealing groove in the process.

4.3. To remove the bulk of the material stuck to the micro-reactor walls, scrub the inside cavity of the micro-reactor with solvent-soaked (toluene or dichloromethane) pieces of paper towels. Repeat the process with pieces of emery cloth, preferably coarse grit (#100).

Note: During this process, do not scratch the sealing surfaces. At the end of this step, the metallic shine of stainless steel inside the micro-reactor cavity should be apparent.

4.4. Remove the material stuck to the flat surfaces of the custom-machined magnet using a piece of emery cloth, preferably coarse grit (#100).

4.4.1. Use a solvent-soaked 1/16" wire to remove the material stuck inside the hole of the custom-machined magnet.

4.5. Use solvent-soaked (toluene, dichloromethane, or acetone) cotton swabs to remove the material stuck to the sapphire window.

4.6. To remove the remainder of the material stuck to the reactor walls, including the sealing surfaces, use solvent-soaked (toluene or dichloromethane) cotton swabs.

Note: The cleanup process is finished when, after scrubbing with a solvent-soaked cotton swab, the cotton swab comes out with negligible traces on it.

Note: However tedious this process might be, this step is important to avoid cross-contamination between experiments.

4.7. Let the micro-reactor air-dry.

5. Image analysis⁹

5.1. Extract information from micrographs pertaining to the mean values of the red, green, and blue (RGB) channels, as well as the corresponding information in the hue, saturation, and intensity (HSI) color space.

Note: The HSI color space is described by cylindrical coordinates, where hue, saturation and intensity correspond to the angular, radial, and vertical coordinates, respectively. The relationships between the RGB values of a pixel and the corresponding HSI values are given by the following equations^{12,13}, where m is the minimum of RGB values, while α and β are the pair of chromaticity coordinates:

$$\alpha = \frac{1}{2} (2R - G - B) \quad \text{Eq. 1}$$

$$\beta = \frac{\sqrt{3}}{2} (G - B) \quad \text{Eq. 2}$$

$$m = \min(R, G, B) \quad \text{Eq. 3}$$

$$H = \text{atan2}(\beta, \alpha) \quad \text{Eq. 4}$$

$$I = \frac{1}{3} (R + G + B) \quad \text{Eq. 5}$$

$$S = \begin{cases} 0 & \text{if } m = I \\ 1 - m/I & \text{if } m \neq I \end{cases} \quad \text{Eq. 6}$$

REPRESENTATIVE RESULTS:

The visual evolution of Athabasca Vacuum Residue is representative of the behavior of asphaltenic heavy crude oil samples and asphaltenic vacuum residue samples under thermal cracking conditions. However, using different samples and/or different temperature, pressure, or reaction conditions can give rise to a wide variety of phase behaviors. Micrographs

corresponding to the thermal cracking experiment on an Athabasca Vacuum Residue sample at final set-point conditions of 435 °C and P_{atm} (N_2) are given in Figure 3, while Figure 4 shows the evolution of temperature during the experiment.

At room temperature, this sample is a pasty solid; thus, the sapphire window is mostly not wetted by the sample and is in contact with gas (in this case, N_2). An air/sapphire interface yields a much brighter reflection than an oil/sapphire interface, so the appropriate illumination and exposure settings to image a liquid sample will always yield white regions if the sapphire surface is in contact with gas. At a higher temperature (> 150 °C), the sample becomes fluid enough to flow and wet the window surface. Small mineral solids inside the sample, which can be identified by small bright elements (Figure 3 A), can serve as an indicator of the stirring efficiency. As the sample is heated to higher temperatures, the images brighten correspondingly, with no color change as long as no significant reaction is taking place. Thermal cracking reactions in asphaltenic vacuum residues cause color and brightness changes that correspond to the chemical transformation of the sample. At extended reaction times, the formation of domains of anisotropic carbonaceous phase (mesophase) can be detected as stationary heterogeneities on the window (Figure 3 D).

An image analysis of the series of micrographs is shown in Figure 5 and Figure 6, which show the evolution of brightness intensity and color with reaction time, respectively. At very early reaction times, the increase in image brightness follows the evolution of the temperature inside the reactor. As the temperature inside the reactor approaches the 435 °C set-point, thermal cracking reactions in the Athabasca Vacuum Residue become prevalent. Thermal cracking reactions in Athabasca Vacuum Residue induce a brightness change in the sample that follows a decreasing exponential trend. In the same period, the color of the sample remains stable in the first part of the reaction before undergoing a shift towards a blue color. The formation of mesophase has the effect of increasing the overall brightness intensity and enhancing the blue color shift⁹.

FIGURE LEGENDS:

Figure 1. Photographs of the micro-reactor, held upside-down by a clamp. Pre-loading arrangement, with the bottom face opened (A). The loaded and sealed micro-reactor (B).

Figure 2. Examples of preferable fields-of-view, as outlined by red rectangles, with respect to the inner surface of the sapphire window.

Figure 3. Micrographs taken during a thermal cracking experiment on Athabasca Vacuum Residue with a condition set-point of 435 °C and P_{atm} (N_2) after 0 min (A), 25 min (B), 50 min (C), and 80 min (D). The length of the red scale bar corresponds to a distance of 100 μm .

Figure 4. The temperature inside the reactor during a thermal cracking experiment on Athabasca Vacuum Residue with a set-point of 435 °C and P_{atm} (N_2).

Figure 5. Evolution of the brightness intensity (I) of the micrographs taken during a thermal cracking experiment on Athabasca Vacuum Residue under 435 °C and P_{atm} (N_2), normalized by the brightness of the micrograph taken at 350 °C.

Figure 6. Evolution of the hue and saturation (H and S in polar coordinates) of the micrographs taken during a thermal cracking experiment on Athabasca Vacuum Residue under 435 °C and P_{atm} (N_2).

Figure 7. Evolution of the brightness intensity (I) of the micrographs taken during a thermal cracking experiment on Cold Lake bitumen under 435 °C and P_{atm} (N_2), normalized by the brightness of the micrograph taken at 350 °C. The data points outlined in red correspond to pictures taken with an overheated objective.

Figure 8. The main incident rays (blue arrows) and reflected rays (red arrows) involved in the illumination of a sample through a window.

Figure 9. Micrographs taken during a thermal cracking experiment on Athabasca Vacuum Residue with a condition set-point of 435 °C and P_{atm} (N_2) after 0 min (A), 25 min (B), 50 min (C), and 80 min (D), taken using bright-field microscope settings instead of cross-polarizers. The length of the red scale bar corresponds to a distance of 100 μm .

Figure 10. Micrographs taken during a thermal cracking experiment on Athabasca Vacuum Residue with a condition set-point of 435 °C and P_{atm} (N_2) after 0 min (A), 25 min (B), 50 min (C), and 80 min (D), taken using a YAG window instead of a sapphire window. The length of the red scale bar corresponds to a distance of 100 μm .

Figure 11. Examples of varied phase behaviors observed during thermal cracking (A, B, and C) and coal liquefaction (D) experiments. Gas-liquid-anisotropic solid (A), liquid-isotropic solid (B), liquid-anisotropic semi-solid (C), and liquid-liquid crystal (D) multiphase systems. The length of the red scale bar corresponds to a distance of 100 μm .

Figure 12. Evolution of the brightness intensity (I) of the micrographs taken during a hydroconversion experiment, normalized by the brightness of the micrograph taken at 350 °C. The hydroconversion experiment was carried out on a heavy vacuum gasoil sample under 420 °C and 15 MPa (H_2), with 12.3 wt.% Ni/Mo catalyst.

DISCUSSION:

Critical steps within the protocol

The first critical step in the protocol is ensuring the integrity of the metal-to-sapphire seal, especially if the experiment is to be carried out under pressure. Thus, the parallelism, the smoothness, and the cleanliness of the sealing surfaces should be carefully inspected, and the leak tests should be thorough. Since the modulus of rupture of sapphire is a decreasing function of temperature¹⁴, thicker sapphire windows should be used for work at high pressure and high

temperature. As a guideline, 8 mm-thick sapphire windows are used in our experiments aiming to emulate hydroconversion conditions (400-450 °C and 16 MPa H₂).

The second critical step relates to obtaining high-quality images, which require the bright illumination of the sample; a clean train of optics; adapted microscope settings (wide iris aperture and long working-distance objectives); and the proper alignment between the microscope objective, the reactor window, and the supporting platform.

For the quantitative analysis of image information, it is critical not to overheat the microscope objective while performing the observations. The method described in step 3.6.1 of the Protocol prevents such overheating. If the operator omits to remove the objective from underneath the reactor between two snapshots taken one min apart, the second picture will appear noticeably brighter as a result. To illustrate this issue, the data points outlined in red on Figure 7 correspond to pictures where the operator had left the objective underneath the reactor in the previous min.

Lastly, it is important to clean the reactor thoroughly between experiments in order to avoid cross-contamination.

Modifications and troubleshooting

Poor data quality generally results from a poorly controlled operating variable (temperature, pressure, or stirring), or a problem in the train of optics. Possible problems in the train of optics include: poor illumination; a small iris aperture; misaligned cross-polarizers; dirty mirrors, filters beam splitters, or objectives; a misaligned reactor or supporting platform above the objective; a dirty or scratched sapphire window; a maladjusted field of view; overheated objectives; and out-of-focus objectives.

Limitations of the technique

For present configuration of the experimental setup, the main limitation of this technique is the lack of ability to reproduce the same level of image brightness across different experiments. In addition to the cleanliness and the alignment of the train of optics, image brightness was found to be very sensitive to the positioning and tilt of the reactor over the objective, which currently are not tightly controlled parameters. However, normalizing the image brightness of a series of micrographs in a given experiment by the image brightness of a micrograph taken at a reference temperature within the same series provides a satisfying workaround, as it yields reproducible data.

Significance of the technique with respect to existing/alternative methods

The combination of cross-polarizers in the train of optics of an inverted microscope with a reactor window made of sapphire allows the observation of high-contrast images of the sample *in situ*. When shining light on an opaque sample through a window, two main reflections are involved, as shown in Figure 8: the reflection of the light on the outside surface of the window in contact with air, and the reflection of the light on the inside surface of the window in contact

with the sample. The intensity of the reflection at each interface is given by the following equation¹⁵:

$$R = \frac{(n_2 - n_1)^2 + \kappa_2^2}{(n_2 + n_1)^2 + \kappa_2^2} \quad \text{Eq. 7}$$

where indices 1 and 2 refer to the media located before and beyond the interface, respectively; n describe refractive indices; and κ is the extinction coefficient. In air/sapphire and sapphire/oil reflections, the contribution of the extinction coefficient to the reflection can be neglected. Considering the refractive index of sapphire in the C-axis direction (extraordinary ray) as 1.765 (average in the 380-700 nm range)¹⁶, the intensity of the first reflection at the air/sapphire interface is about 7.7% of the incident light. Since most oil samples have refractive indices ranging from 1.45 to 1.6¹⁷, the intensity of the second reflection at the sapphire/oil interface can be considered as less than 0.9% of the incident light. On first approximation, the air/sapphire reflection is at least more than 9 times brighter than the sapphire/oil reflection. Hence, when observations are made under bright-field settings (using unpolarized light), visuals of the sample are outshined by the air/sapphire reflection. To illustrate this issue, micrographs taken under bright-field settings during the thermal cracking experiment on an Athabasca Vacuum Residue sample at final set-point conditions of 435 °C and P_{atm} (N_2) are presented in Figure 9 (the microscope lamp voltage was reduced to 10 V and the camera exposure was reduced to 25 ms to avoid blow-outs) [place Figure 9 herewith]. As can be seen by comparing Figure 9 to Figure 3, the presented method for observing the sample using cross-polarized light and a sapphire window has the advantage of yielding high-contrast images that are able to describe isotropic media.

As the light is reflected at the air/sapphire interface, its polarization plane does not change. Thus, the cross-polarizer setting cancels this reflection before it hits the CCD camera. As light travels through sapphire, however, its polarization plane rotates because of the sapphire birefringence. This phenomenon ultimately allows sample imaging, even if the oil sample itself is isotropic and the polarization plane of the light does not change upon the sapphire/oil reflection. If the cross-polarizer setting is used in combination with an optically isotropic window (such as fused silica or yttrium-aluminum-garnet, YAG), then only an anisotropic medium (changing the polarization plane of the light at the window/sample interface) and depolarized fluorescence can be viewed. Figure 10 presents micrographs taken during a thermal cracking experiment on an Athabasca Vacuum Residue sample at final set-point conditions of 435 °C and P_{atm} (N_2) using the cross-polarizer setting and a 4 mm-thick YAG window [place Figure 10 below].

In comparison to the presented technique, the top-down, hot-stage configuration used in other works^{11,18} has the disadvantage of featuring a gap of gas between the inner surface of the reactor window and the liquid sample. In such a configuration, using a sapphire window would produce images dominated by the brightness of the sapphire/gas reflection, very similar to the use of bright field with an inverted microscope. Thus, the operators of the top-down hot stage

used a reactor window made of YAG, which only allows for the observation of anisotropic material, as explained previously.

The optical properties of a sample may evolve as it undergoes a change in temperature, pressure, or reaction time. The formation of a multiphase system can be characterized by the formation of heterogeneity on the window surface. Figure 11 shows examples of gas-liquid-anisotropic solid, liquid-isotropic solid, liquid-anisotropic semi-solid, and liquid-liquid crystal multiphase systems

[place Figure 11 below].

For homogeneous, single-phases systems, changes in the brightness and color of the sample can be related to physical and chemical properties. Following Equation 7, changes in sample brightness are attributed to changes in the refractive indices. In particular, the greater the difference in refractive indices between the sample and the sapphire, the brighter the reflection. For instance, as a heavy oil sample is heated to temperatures below 300 °C, the refractive index of the oil decreases while the refractive index of the sapphire slightly increases, yielding brighter images. During isothermal cracking reactions of vacuum residue samples, images undergo an exponential decrease in brightness; this is attributed to an increase in refractive index due to an increase in aromaticity and density. Conversely, hydroconversion reactions at constant temperature produce a gradual increase in sample brightness, which corresponds to a decrease in refractive index following a decrease in the density of the sample.

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 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 blue⁹.

Future applications or directions after mastering this technique

While our studies involving the use of this setup have been primarily related to phase separation phenomena during visbreaking and hydroconversion of heavy petroleum samples in downstream operation, the technique could be applied to the investigation of other phase separation mechanisms occurring in oil processing units and lines (wax crystallization, demulsification, etc). More generally, this technique could be applied to any system where tracking the optical properties of a sample *in situ* is of great importance.

Our current research efforts are focused on establishing more relationships between the spectral properties and the physical properties (solubility in particular) of petroleum samples. At present, the spectral information contained in images is limited, since it is expressed in three

color channels (RGB). Therefore, the most promising development of this technique lies in the implementation of hyperspectral characterization.

ACKNOWLEDGEMENTS:

The authors acknowledge Daniel Palys for supplying Figure 12 and for his assistance in managing laboratory supplies.

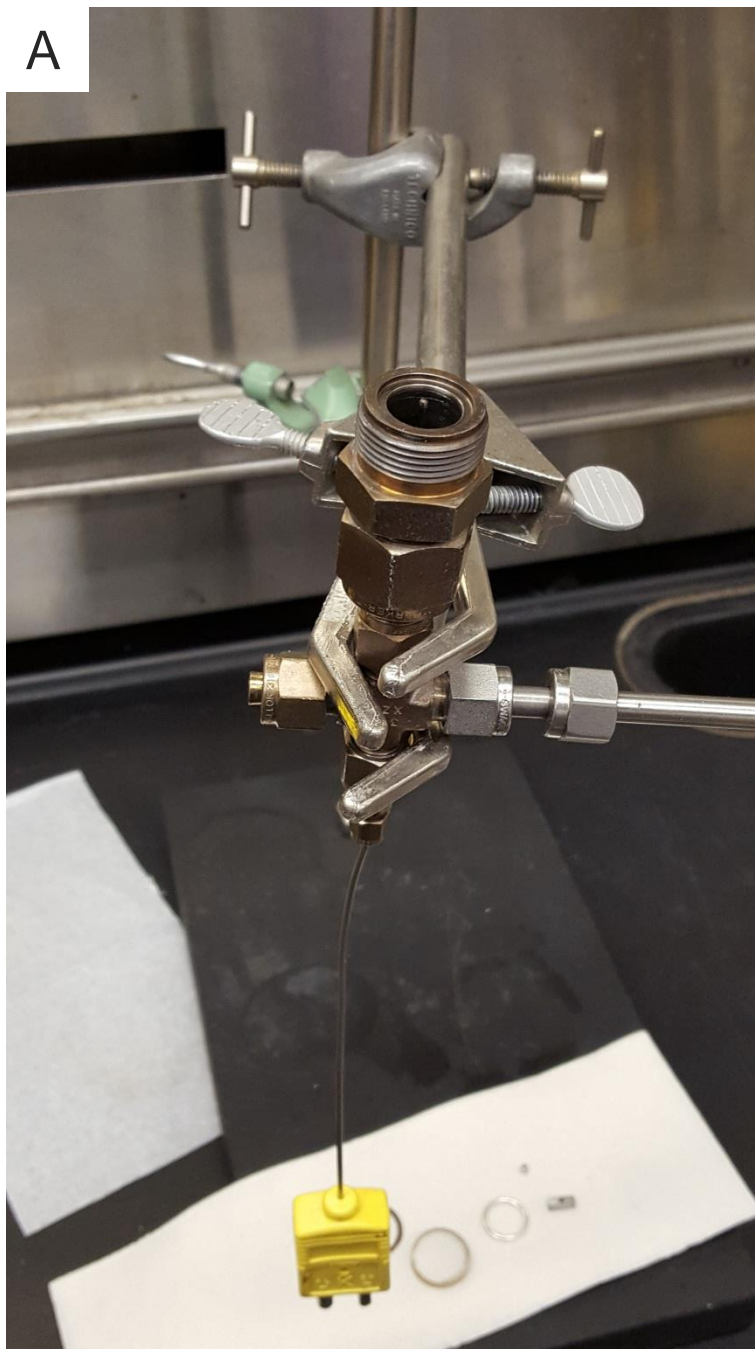
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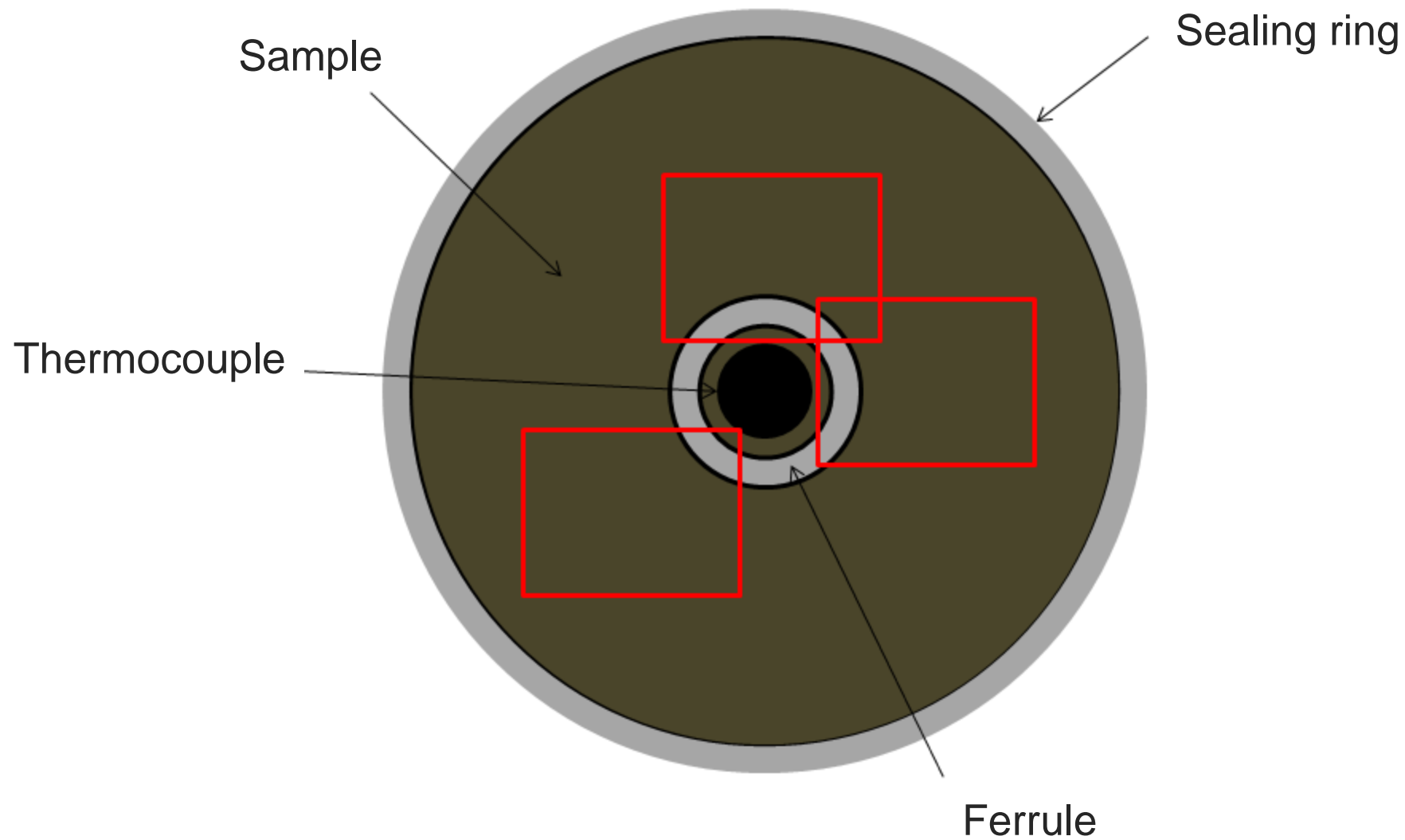
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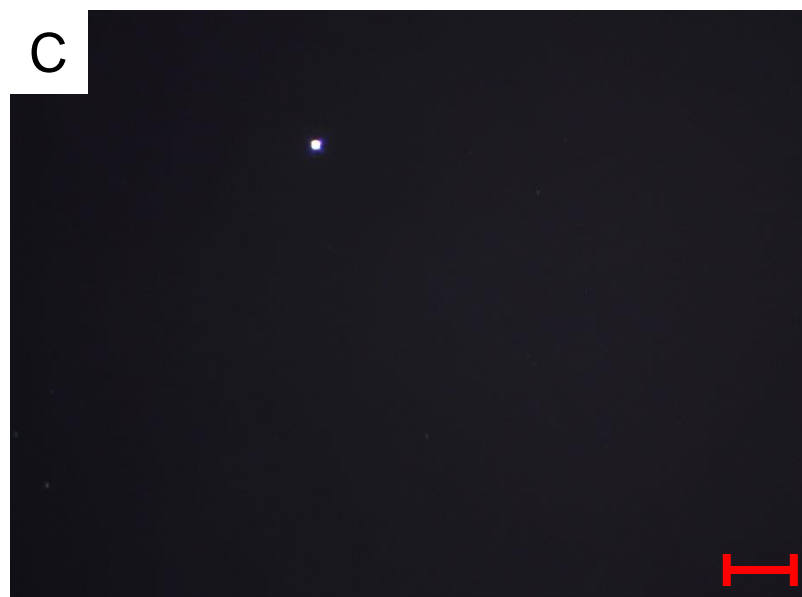
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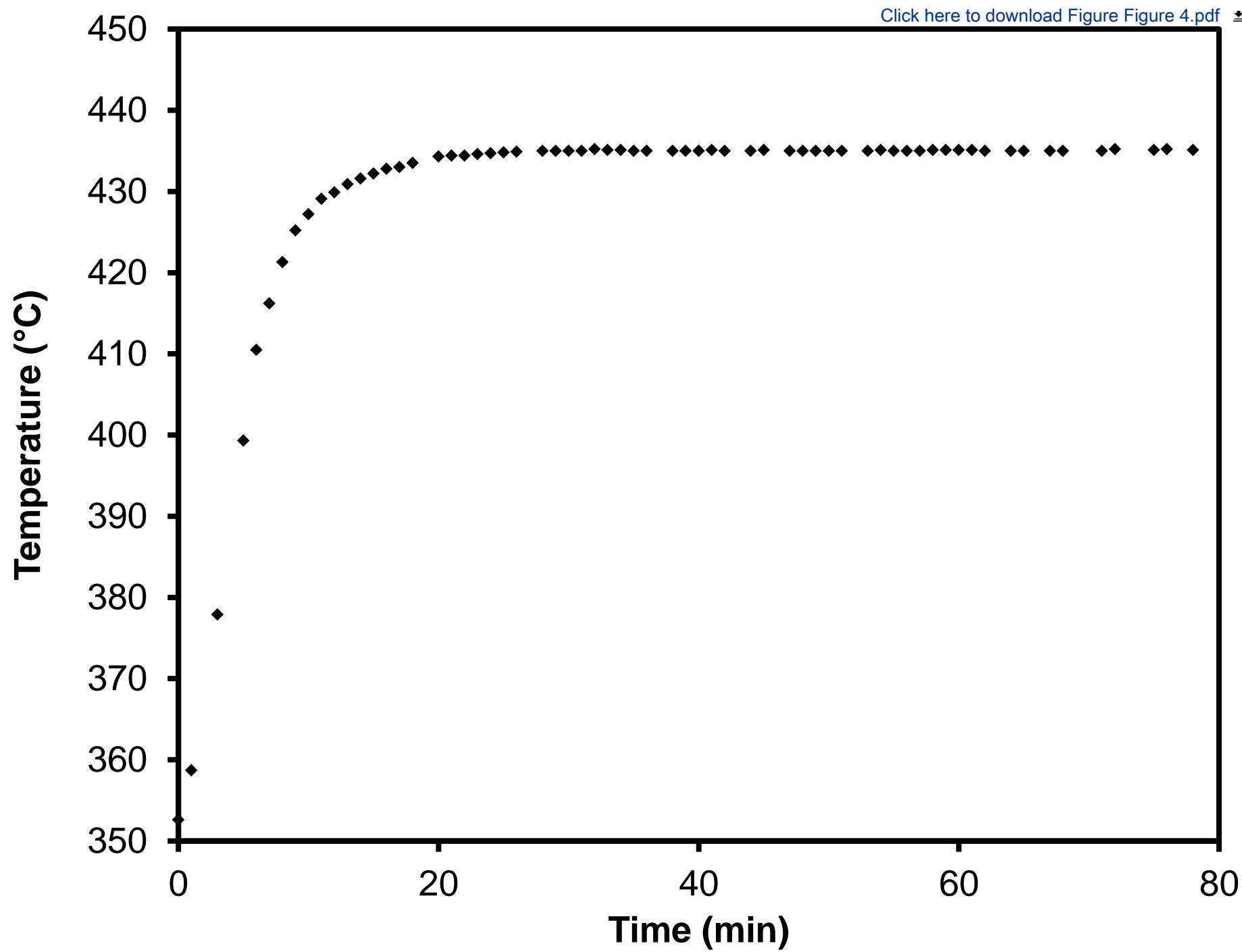
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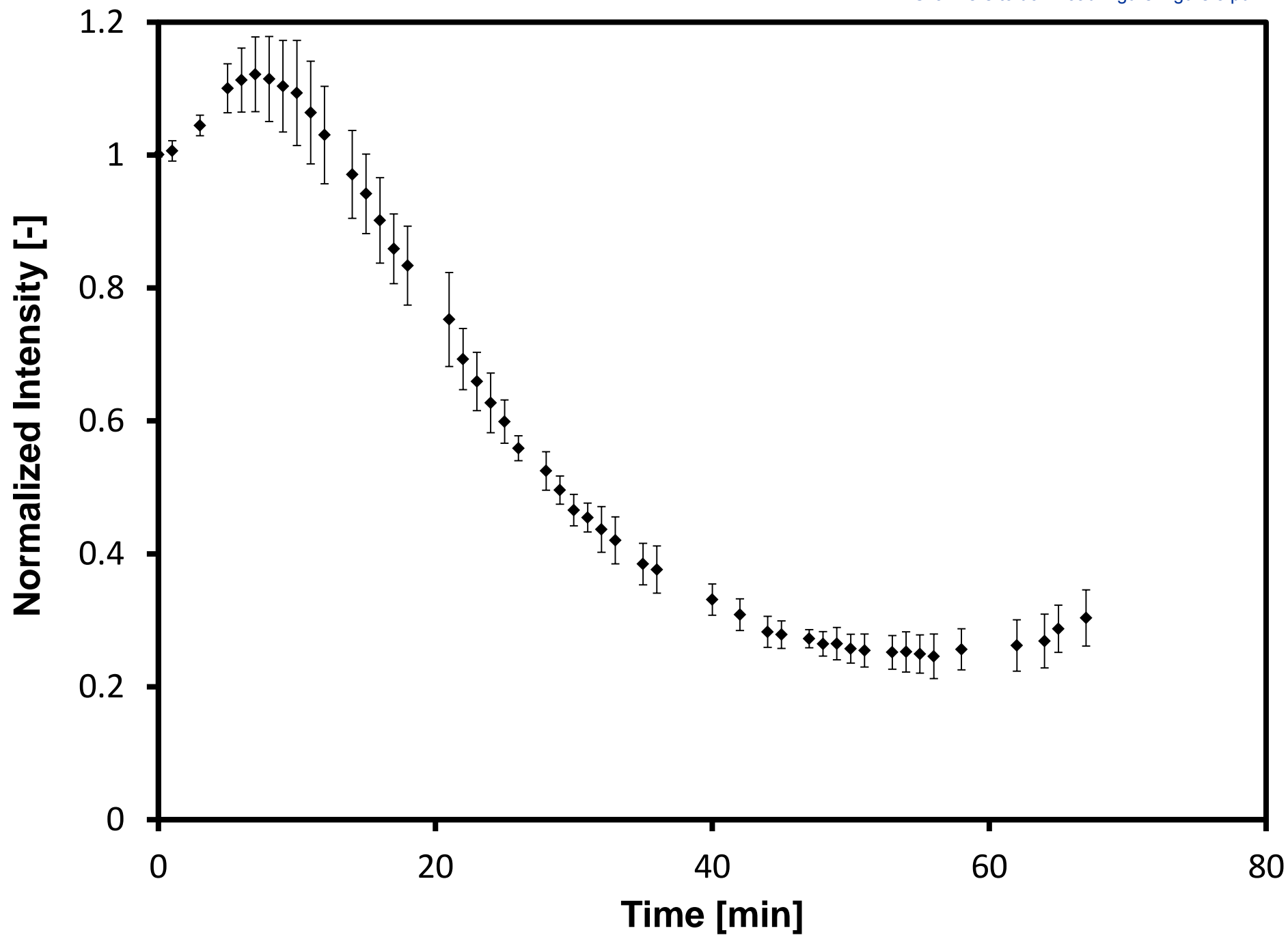
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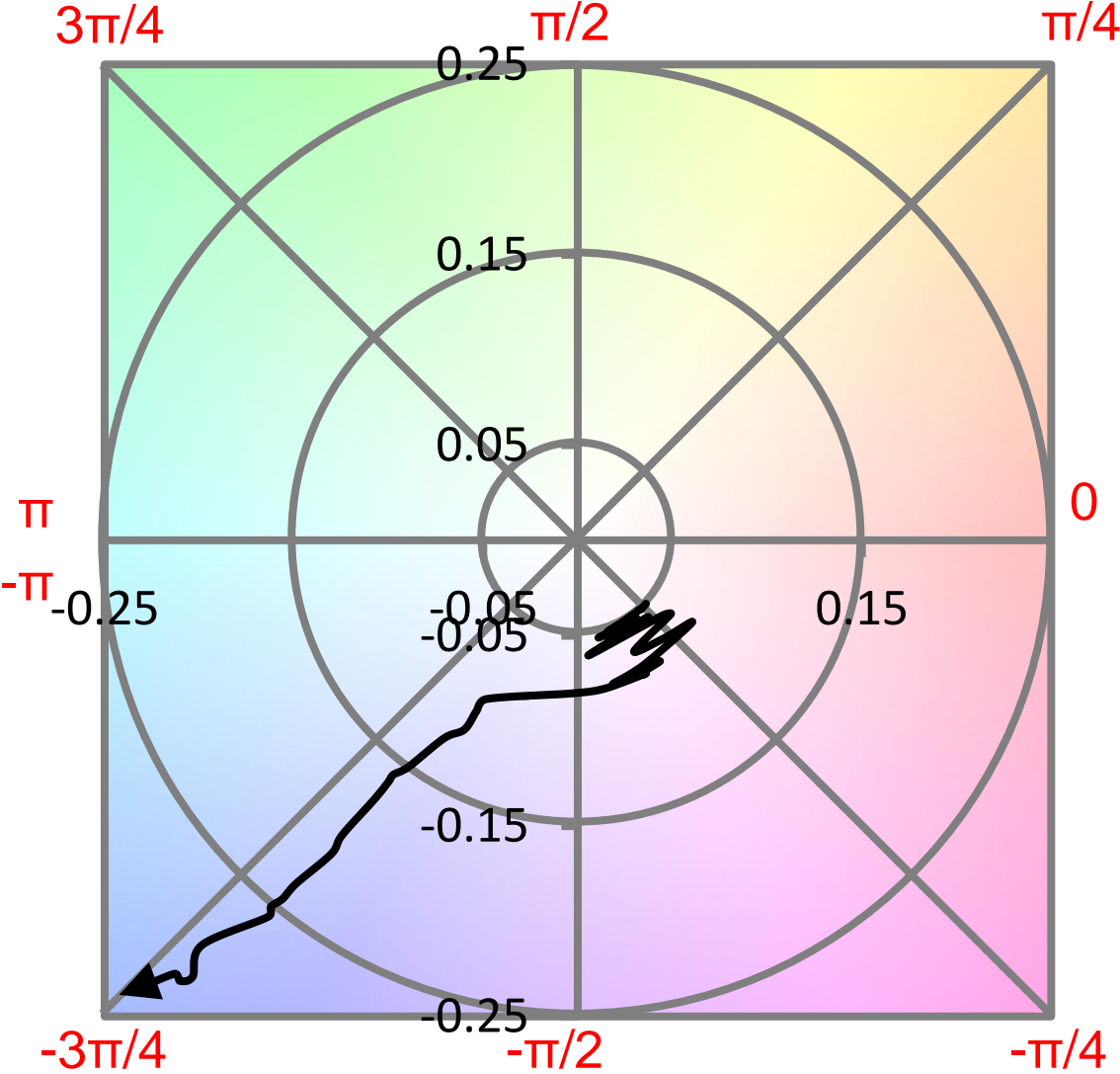


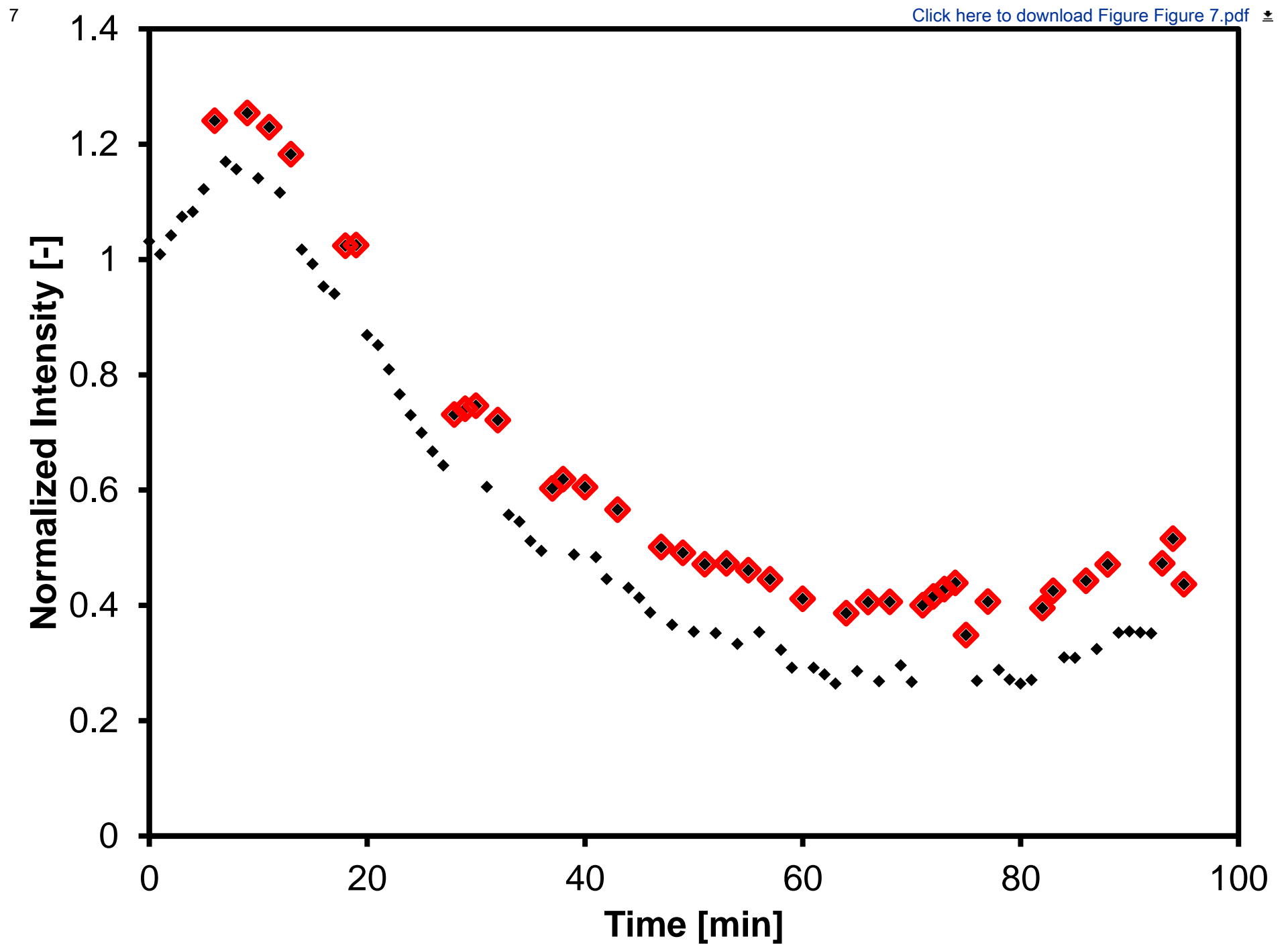


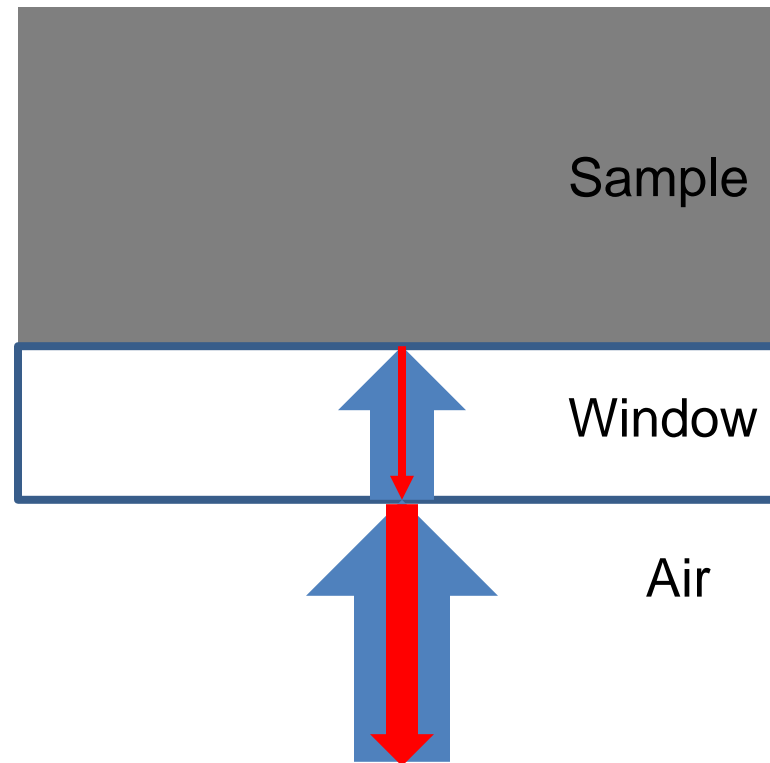












A



B



C



D



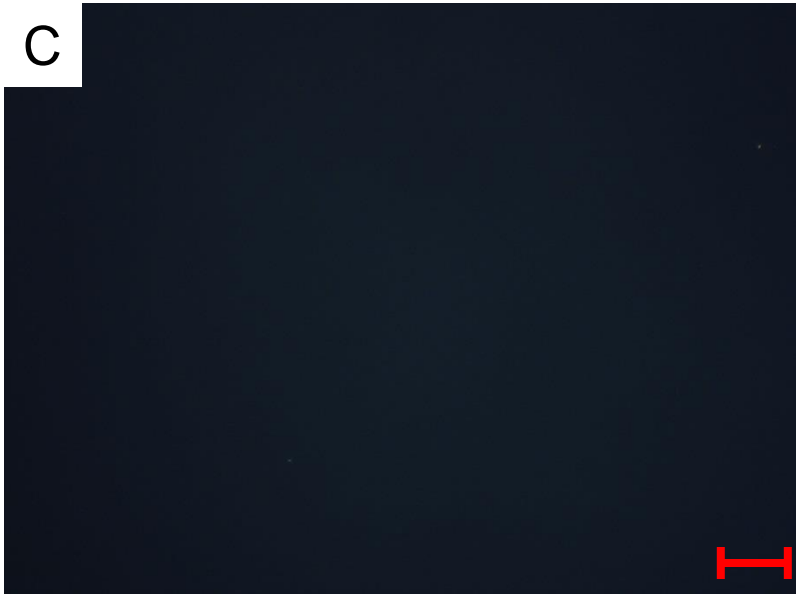
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B

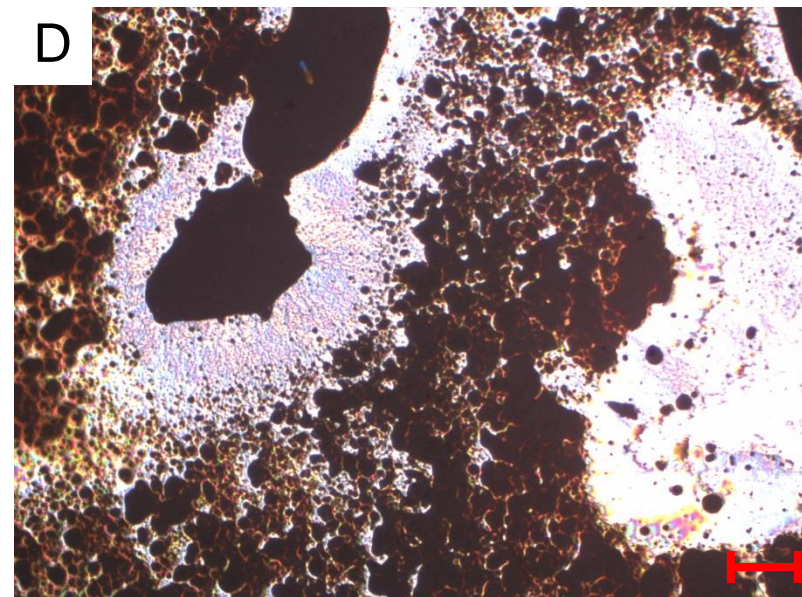


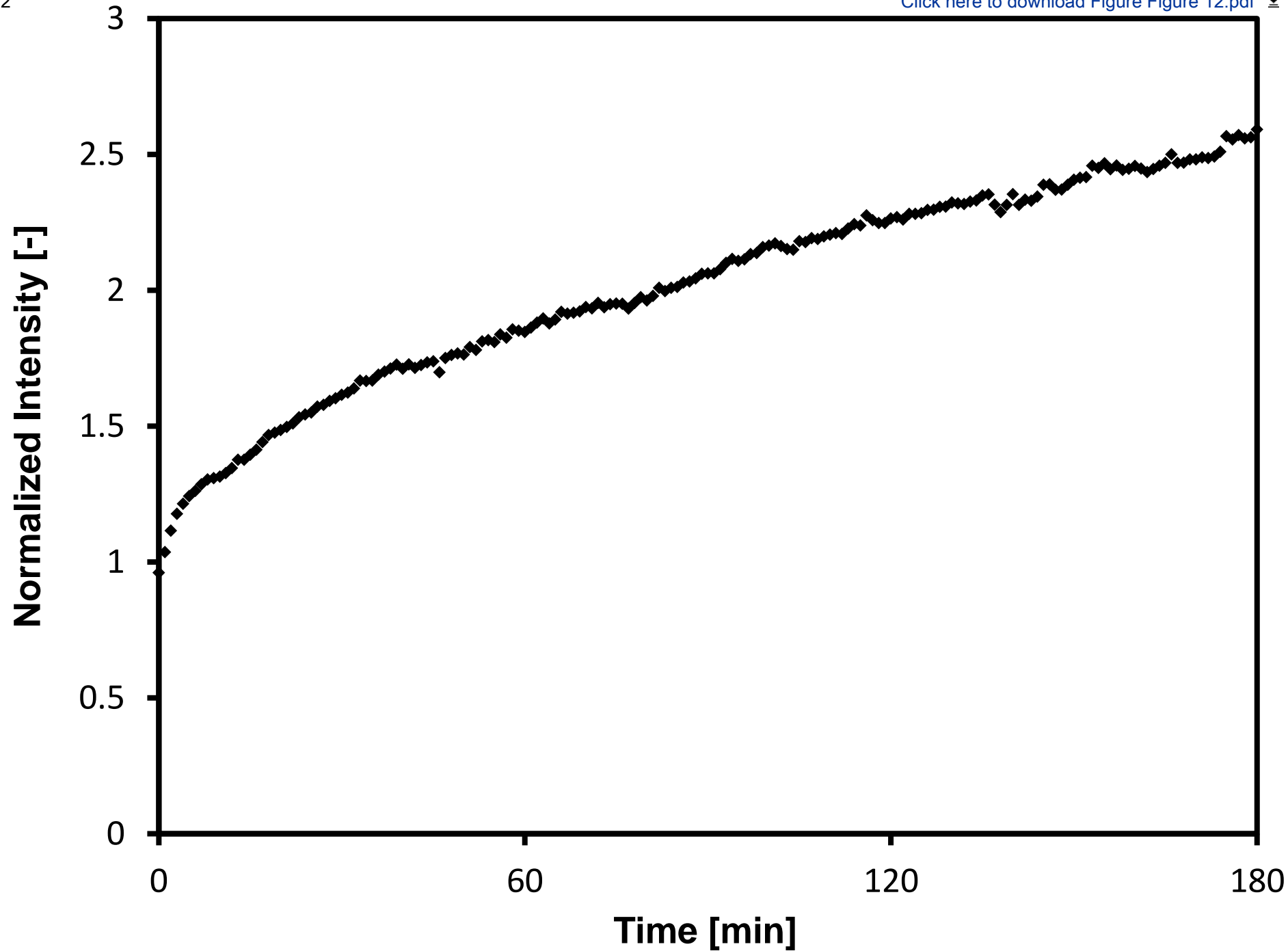
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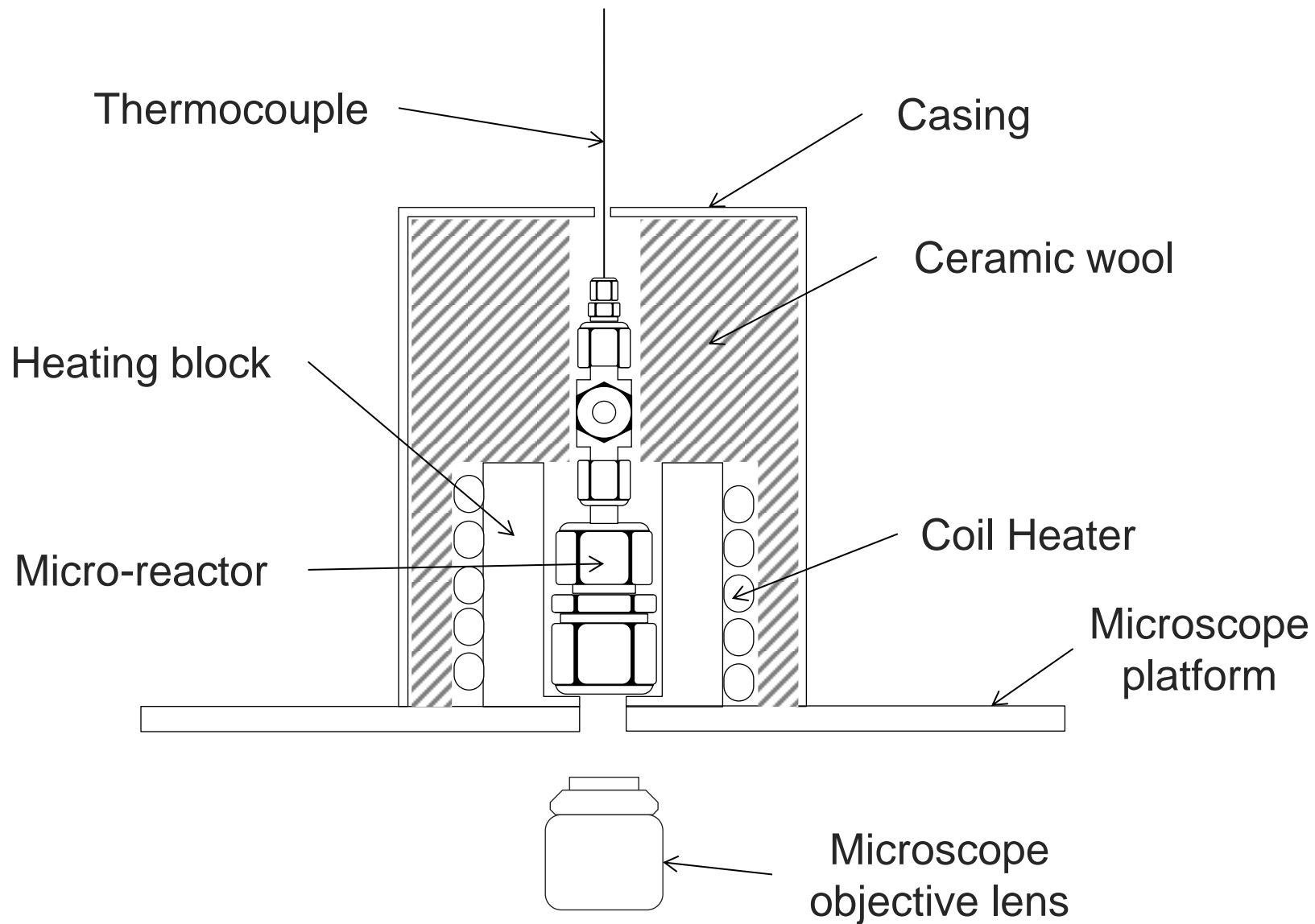


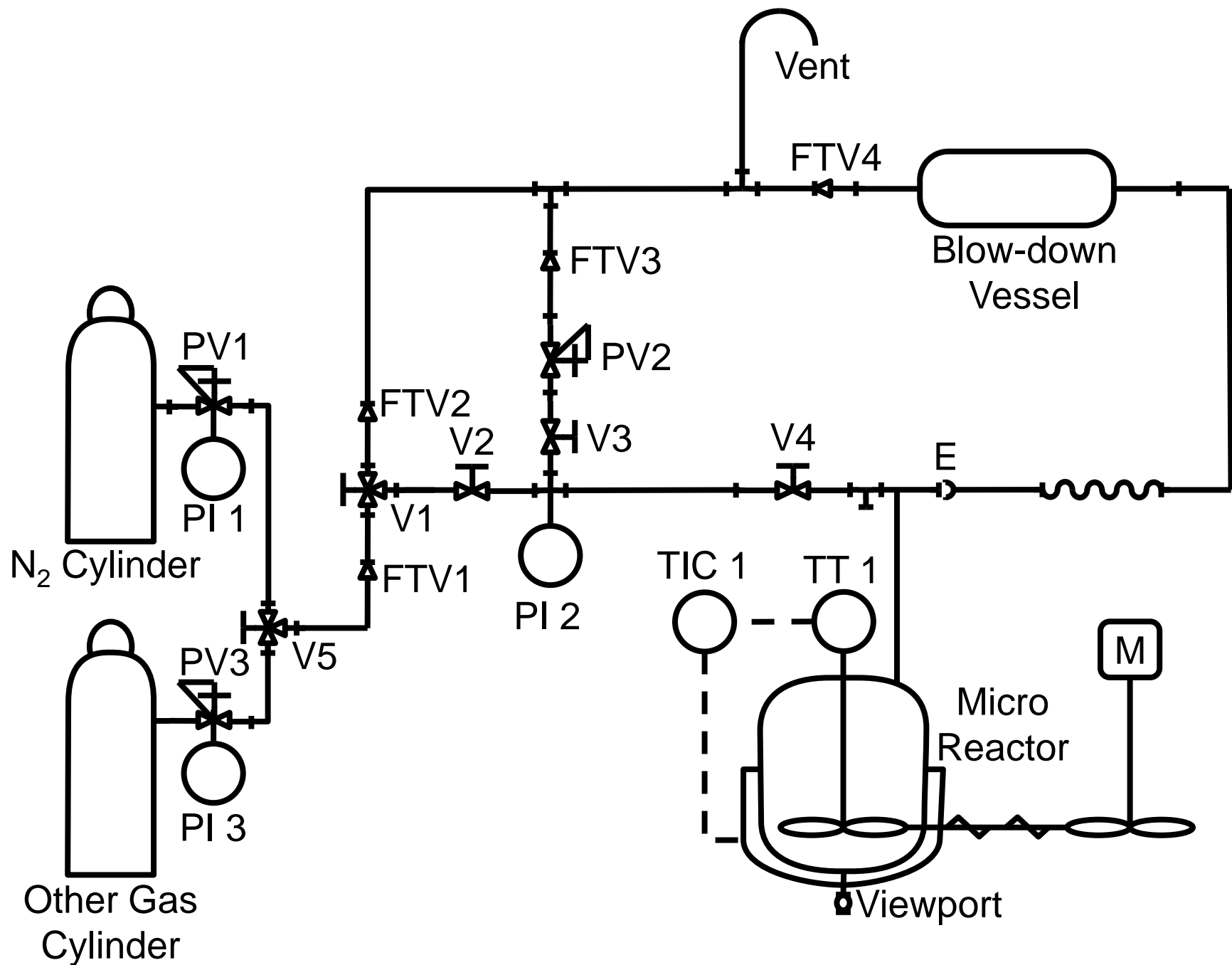
D











Name of Material/ Equipment	Company	Part Number	Comments/Description
Sapphire window, C-plane, 3mm thick - 20 mm diam., Scratch/Dig: 80/50	Guild Optical Associates		
C-seal	American Seal & Engineering	31005	
Type-K thermocouple	Omega	KMQXL-062U-9	Inserted for creating a clearance gap between the magnet and the window surface
Ferrule (1/16")	Swagelok	SS-103-1	
Coil Heater	OEM Heaters	K002441	
Temperature controller	Omron	E5CK Axio	
Inverted microscope	Zeiss	Observer.D1m	Require cross-polarizer module
Toluene, 99.9% HPLC Grade	Fisher	Catalog # T290-4	Harmful, to be handled in fume hood
Methylene chloride, 99.9% HPLC Grade	Fisher	Catalog # D143-4	Harmful, to be handled in fume hood
Acetone, 99.7 Certified ACS Grade	Fisher	Catalog # A18P-4	



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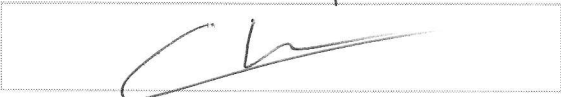
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Object: Post-peer-review Revision of Manuscript to the Journal of Visualized Experiments

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 micrographs 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, hydroconversion.... " 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.

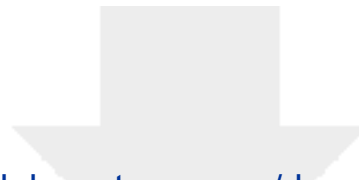
Minor Concerns:

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



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Supplemental File (as requested by JoVE)
Supplemental file_setup description.docx

