Stabilizing Microscope Images of Heated Samples

Holden Kowitt
Goldhaber-Gordon Lab, Stanford University, Stanford, CA, USA
(Dated: February 4, 2024)

When a sample observed under a microscope is heated above room temperature in ambient conditions, the image appears to shake due to convection in the air between the sample and the objective lens. This effect becomes dramatic above ∼150 °C, a temperature range relevant for new methods of stacking atomically-thin van der Waals materials. Here we implement and evaluate two interventions – a transparent baffle and a fan – to reduce this shaking. Over a range of sample temperatures from 25 °C to 300 °C, the combination of the fan and baffle together was most effective, but either one alone significantly reduced shaking in the images.

I. INTRODUCTION

To form multilayer stacks of atomically-thin materials such as graphene, polymer-based "stamps" are typically used to pick up successive layers. These stamps are held in place on a series of two independent micro-manipulators, providing translational and rotational degrees of freedom. Recently, a new type of stamp has been introduced, based on a flexible cantilever with a thin coating of gold as an adhesive [1]. These polymer-free stamps are expected to introduce far less contamination during assembly than their polymer counterparts, allowing formation of multilayer stacks free of bubbles and wrinkles. A challenge in using these stamps is the requirement to heat substrates to temperatures as high as 300 °C, far above the 80 °C to 120 °C used for polymer-based stamps. We have found that such high temperatures destabilize the microscope images, making it difficult to align the stamp with thin flakes on a substrate to the required accuracy on the order of 1 μm.

This project aims to reduce the shaking in the image mentioned above and to protect the components of the stacking system, notably the microscope objective, from excessive heating. We began by adding thermal insulation around the heating element to limit the exposed heated surface, reducing convection. Then we added a transparent baffle to physically block the hot air, and a fan to move excess hot air out of the system.

Previously, our lab had obtained polymer-free stamps from their developers, but the shaking had prevented extensive testing of their efficacy.

II. DESIGN PHILOSOPHY

We have observed the destabilization of the microscope images at temperatures above ∼100 °C. Convection within the air creates hotter and cooler pockets with resulting differences in index of refraction. The movement of the air causes light from the flake that reaches the lens of the microscope to take different paths on short timescales, destabilizing the image. This effect is analogous to distortion of astronomical images based on changing indices of refraction in the atmosphere. In astronomy, adaptive optics [2] has proved effective, but we sought a simpler solution.

We decided to surround the sample with thermal insulation, to limit the amount of air heated. We also used a fan to push the heated air away from the space between the sample and the objective, replacing it with more uniform cooler air. Finally, we designed a transparent baffle to place between sample and objective, limiting the distance over which convective currents can form. We examined the effect of adding or removing the fan or baffle. However, since the thermal insulation is difficult to install and remove we left it in place throughout.

III. METHODOLOGY

A. Design of system

The main purpose of the baffle (Fig 1) was to maintain the usability of the system while reducing shaking as much as possible. Different designs were created using 3D CAD software. The baffle was connected to the micro-manipulator stage that holds the stamps, so that the baffle can achieve closer blocking compared to being fixed at the maximum height of the stage. The final design of the baffle system consists of a 1 mm thick 3 inch optically flat square BK-7 glass sheet, a two-piece sliding aluminum frame, and a new stage that included a railing and dowel pin holes to help support the frame (see appendix, Figures 4–6). The frame is easily removable, being only held in with a sliding rail so users can set up their materials and stamps without being limited by working around the baffle. The size of the baffle was chosen as the largest readily-available optically flat glass that would fit without colliding with other elements of the pre-existing stacking setup. Designing for the largest size available minimizes the amount of air that could flow around the baffle. Finally, the frame was designed to be two pieces to allow for ease of machining.

The fan was a small 6 inch desktop fan, mounted on a separate table about two feet from the stacking setup. Having the fan so far away is a compromise between

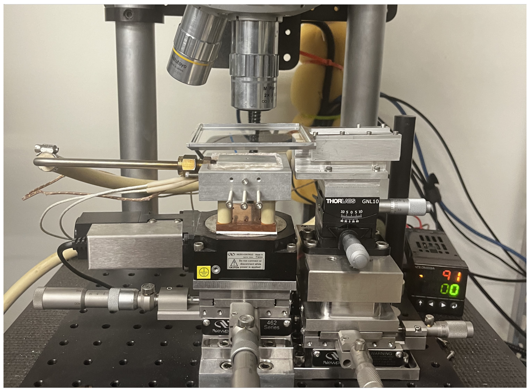

FIG. 1. Photograph of the full stacking setup. At the top is the objective lens housing of the microscope, then below is the sliding frame and glass baffle. The stamp stage is connected to the frame for the baffle. Below the baffle is the aluminum hotplate. Surrounding the hotplate is aerogel insulation and an aluminum container for the insulation.

having enough airflow to disrupt vertical convection and limiting forced-air convective cooling of the hot plate. To avoid vibrations in the microscope, the fan is not mounted on the same table as the microscope.

A layer of aerogel insulation contained within an aluminum rim was added around the hot plate to both limit unnecessary heat from entering the air, and to allow for the hot plate to achieve high temperatures easily with the fan running.

B. Video collection and analysis

Data was collected at 25 °C, 125 °C, 175 °C, 225 °C, and 300 °C. At each temperature, a baseline using neither the fan nor the baffle was recorded. Then data was taken using the fan, the baffle, and both the fan and baffle together. After the hot plate temperature reading stabilized to within one degree of the target temperature, a video recording of the microscope image was taken for a one-minute period without disturbing the setup. Then temperatures of various components were measured using a temperature probe. Then the next configuration was arranged, and the temperature was allowed to stabilize. After all four configurations were evaluated at one temperature, the hot plate was adjusted to the next temperature, and all measurements were repeated.

For the analysis of the video clips, the third ten seconds (t=20 to t=30) of each clip was selected and analyzed using a point tracking software [3]. Ten seconds was chosen to allow the software to process in a reasonable time. The video tracking software tracks a single point across each frame of a video clip, and records the coordinates of that point in x and y.

The figure of merit for the analysis is the radial root mean squared (RMS) deviation of the locations of the points tracked, calculated from the x and y deviation of each point's location from its mean within the dataset in question.

IV. RESULTS

A. Movement of Microscope Images at High Temperatures

The clearest trend in the data is that each intervention to mitigate the shaking has a large effect. Using the fan alone or the baffle alone are comparable, whereas using both together provided the best overall improvement. The RMS motion for all four configurations at the various listed temperatures is shown in Figure 2. Additionally as the temperature increases the shaking tends to be worse, as expected. One unexplained observation is the large jump in radial RMS motion between 225 °C and 300 °C, in absence of interventions.

Examining the time series data above room temperature of the baffle only and the fan only, an interesting distinction can be seen (see appendix). The fan-only data tend to show steady jitter, mostly above 1 Hz. The baffle data, by contrast, has more irregular, low-frequency noise, with excursions typically longer than one second. The time series of the combination of the fan and baffle appears like the sum of the individual noises, but with a lower amplitude. Further investigation should include a proper frequency analysis of the jitter by computing the power spectrum.

B. Calculating error for image movement data, and error sources

Analysis of the error was performed on the 300 °C, no-intervention data set because that data set had the largest RMS jitter, suggesting that it would also have the largest error. Only this trial was selected for error analysis due to the intensive computing time required. Four separate points on the microscope image for this clip were tracked, yielding the RMS values of: 2.14 μm, 1.97 μm 1.64 μm, and 2.47 μm. The standard deviation of those four points is 0.34 μm, which is shown on the data point as the error bar. This estimate is justified because the cross-correlation between each of the four points' x and y had Pearson correlation values between 0.61 and 0.91. Nearly all of the four points are "very strongly"

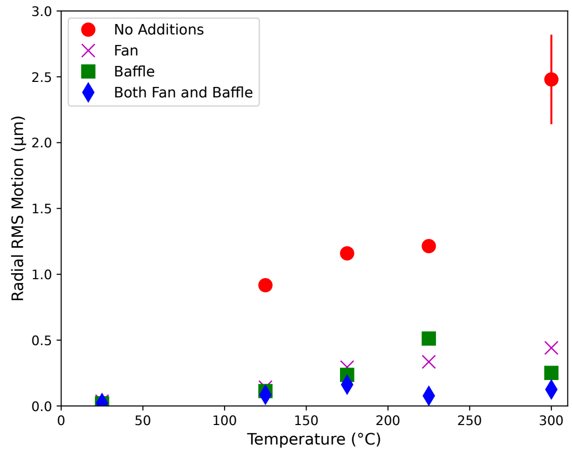

FIG. 2. Effect of temperature on image stability. The horizontal axis shows the hot plate temperature, while the vertical axis plots radial jitter for four cases: original configuration, fan only, baffle only, and combined fan and baffle. An error analysis was performed on the 300 °C original configuration point, and is shown as the vertical bar.

correlated with each other, with one pair just being "strongly" correlated [4]. The strong correlation across the image implies that the image is rigidly shaking rather than having some non-uniform distortion, which justifies the use of the standard deviation as a conservative estimate of the error in the jitter extracted from the four tracked points of the data set.

Within the x and y time series, it appears there are periods of relative calmness and then spikes in movement. These larger movement events are clearly visible in the raw video of the shaking. An example can be seen in the 225 °C trial with only the baffle. Because the frequency of these larger events are unknown, ten seconds may not be sufficient to reliably capture a reasonable number of events. Trials that have these events over-represented are a possible source for error in assigning amount of jitter for a given set of conditions.

Also notable is the gradual drift of the image that appears in the baffle-only dataset at 300 °C. This drift could either be the result of settling of the materials on the wafer, or it could be a thermal expansion within the system. A linear shift in the data would inflate the extracted RMS motion compared to the random shaking which this investigation is seeking to minimize. One possible solution could be applying a linear adjustment to the data, however this was not done.

The RMS values at room temperature differ from the expected RMS of zero for a still image. This jitter is however substantially less than the $0.17\,\mu$m size of a single pixel, so it may not reflect actual image instability and in any case does not interfere with the usage of the stamp setup.

When jittering, occasionally the primary image appears overlaid with a slightly transparent, shifted afterimage. No consistent pattern was observed to the occurrence of these afterimages. The tracking software is not always precise in its tracking of the point when there is an afterimage. This imprecision could lead to error if the tracking is inconsistent in the afterimage recognition between trials.

Another limiting factor is the 20-frames-per-second frame rate, so any jitter above 10 Hz would be lost.

C. Temperature increases of the objective lens

On the trials without any intervention, the objective lens routinely heated significantly more than on the trials with intervention, even surpassing its rated 40 °C safe operating temperature on the 300 °C trial, reaching 41 °C. Using just the baffle was effective in limiting the heating, although at higher temperatures the objective temperature started to rise above room temperature, to a peak of 29 °C. All the trial sets that included a fan were effective at keeping the objective at room temperature through every trial.

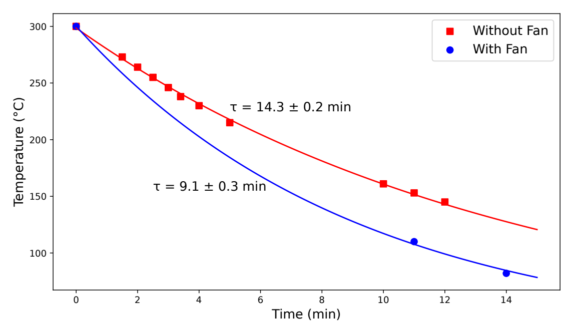

FIG. 3. Effect of fan on cooling time. The horizontal axis shows the time elapsed since the cooling time started, while the vertical axis plots the hot plate temperature

D. Cooling time of full setup

A secondary goal of the project was to decrease the thermal cycle time of the stacking station between uses so it can return to safe handling temperatures quicker and get more use. Using the same fan as the image stabilization, the characteristic time for the cooling from 300 °C reduced by about 35% (Fig 3). This reduction in cooling time adds another benefit to including a fan in the system.

V. CONCLUSION

This work shows that either using just the baffle or the fan produces a significant improvement over nothing, and using a combination of both is more effective than either one. Using both, the image is stable enough to perform assembly of devices up to at least 300 °C. Additionally, using a fan can significantly reduce the cool down time from hot stacks, resulting in better utilization of the stacking setup.

An investigation using a strobe light to eliminate the presence of the afterimages as well as taking longer video samples or taking multiple video samples from the same minute period would reduce the amount of error and yield more precise results.

Further research should include investigations on the effect of the distance from the baffle to the hot plate on the shaking, as well as frequency power spectrum analysis for all of the different setup variations.

Of particular note, the geometry of the flat hot plate results in natural convective currents, described by Rayleigh-Bernard convection cells. The Rayleigh number, Ra, characterizes whether these Rayleigh-Bernard cells can form, if the given number is above a critical value. For a surface bounded between two rigid planes, like the hot plate and baffle, that critical value is 1708 [5]. The Rayleigh number can be calculated using the following:

$$Ra = \frac{g\beta}{\nu\alpha}(T_b - T_u)L^3 \tag{1}$$

where L is the height of the container, g is the acceleration caused by gravity, β is the thermal expansion coefficient, ν is the kinematic viscosity, α is the thermal diffusivity, T_b is the temperature of the bottom plate, and T_u is the temperature of the upper plate.

Using measurements of the top plate temperature when the bottom plate was at 300 °C, and values for air at temperature of 300 °C, equation (1) predicts a critical height of 1.1 cm [6], suggesting that when the baffle is within 1.1 cm of the sample there should be no convection happening at 300 °C, given that Rayleigh cells can't be supported. Given the partially-canceling temperature variations in all the terms in the equation, this distance turns out to be nearly temperature-independent (e.g. 0.94 cm at 100 °C.) We used a sample-baffle distance of 2 cm to accommodate the stamp-holder, but it would be plausible to decrease this distance below 1 cm, which we would expect to decrease the shaking further.

VI. ACKNOWLEDGMENTS

The author thanks Elijah Courtney, Albert Nazeeri, Dr. Mihir Pendharker, Aaron Sharpe, and Chaitrali Duse, all of whom provided invaluable advice and encouragement. This work was performed under the guidance and supervision of Professor David Goldhaber-Gordon. During the writing of this report we learned that a group in Aachen, Germany used only a fan, and found that sufficient for stabilizing their images, albeit at lower temperatures [7].

[1] W. Wang, N. Clark, M. Hamer, A. Carl, *et al.*, Ultraclean assembly of van der waals heterostructures, Nature Electronics **6** (2023).

[2] K. Hampson, R. Turcotte, D. Miller, J. Kurokawa, K. Males, N. Ji, and M. Booth, Adaptive optics for high-resolution imaging, Nature Reviews Methods Primers **1** (2021).

[3] Tracker video analysis and modeling tool for physics education.

[4] H. Akoglu, User's guide to correlation coefficients, Turkish Journal of Emergency Medicine **18**, 91 (2018).

[5] S. Norris, *A Parallel Navier–Stokes Solver for Natural Convection and Free Surface Flow*, Ph.D. thesis, University of Sydney (2000).

[6] Engineering toolbox (2001) [online] available at: https://www.engineeringtoolbox.com.

[7] D. Goldhaber-Gordon, Personal communication (2023).

VII. APPENDIX

Included in the appendix are photographs of the components of the baffle, and the time series data for each configuration at each temperature, along with the radial RMS of each trial.

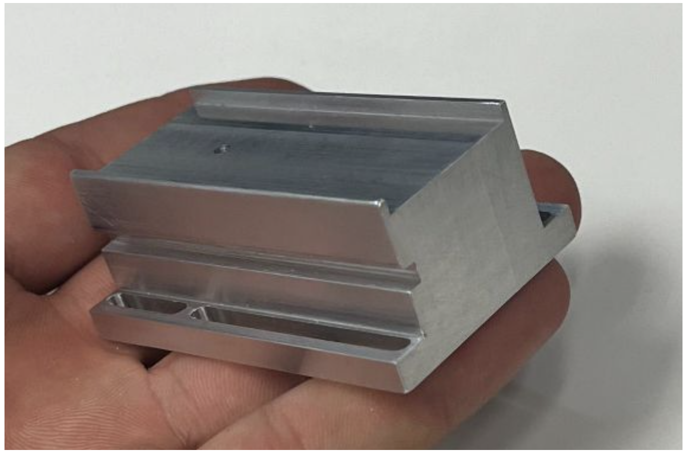

FIG. 4. Stamp stage of baffle system, showing the rail which the baffle frame rides in.

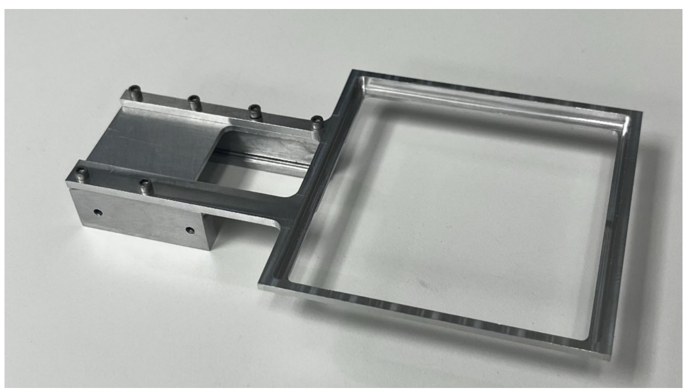

FIG. 5. Baffle frame for baffle system, showing the two part assembly held together with screws visible on the top. Screw holes are shown on the bottom of the frame, included in the design to supplement the railing for stability once slid into place.

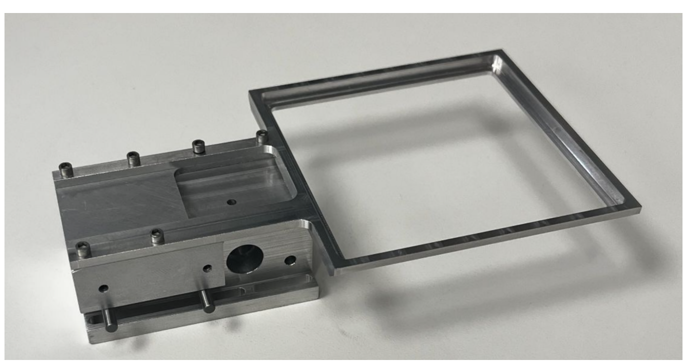

FIG. 6. Baffle system assembled. No glass baffle present, but it would fit into place on the enclosed rims in the frame.

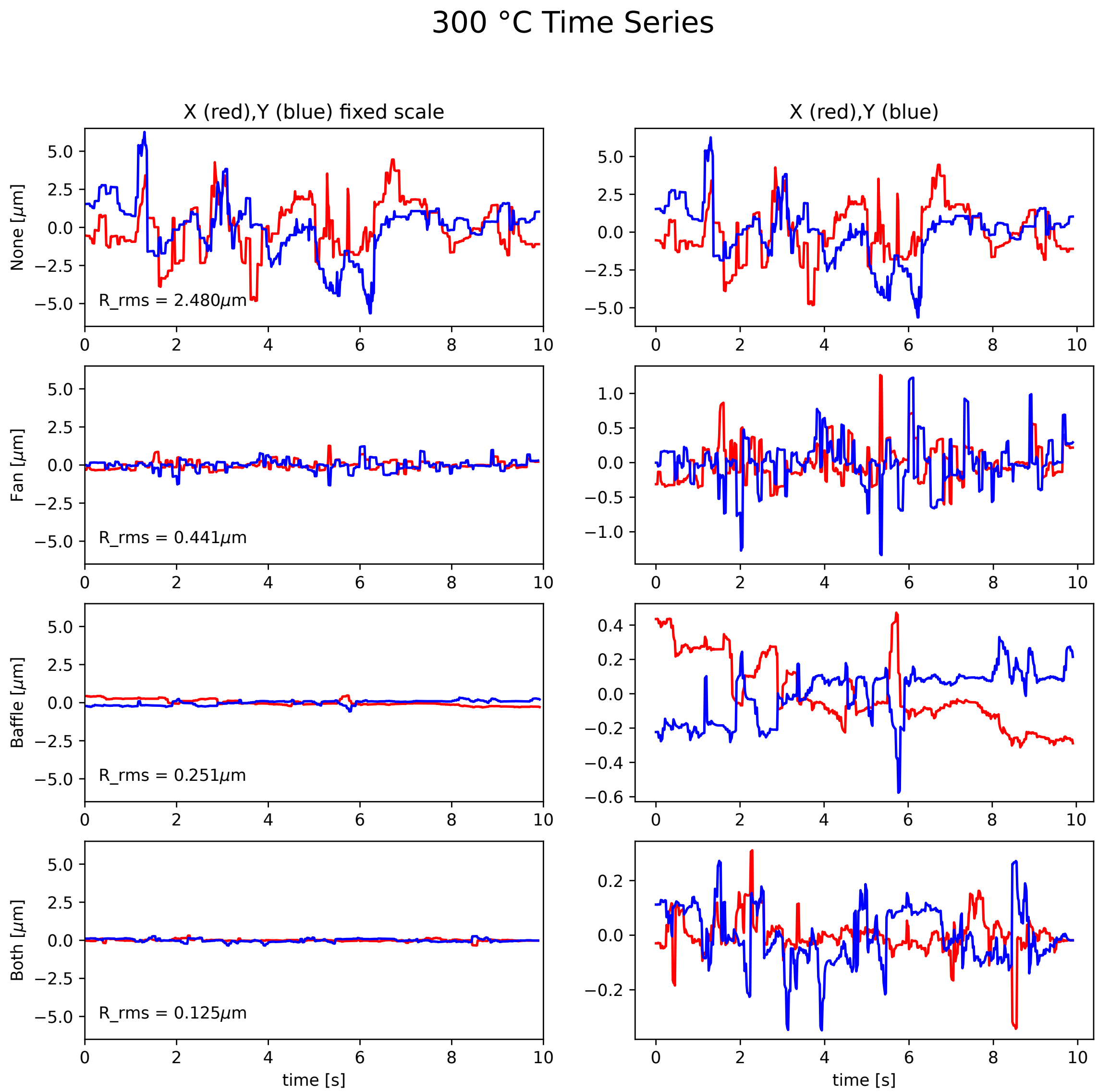

FIG. 7. Time series data of displacement of the tracked point at 300 °C. Note the two columns show identical data–the left side plotted with a fixed vertical scale. From top to bottom, the panels show no intervention, fan only, baffle only, and both fan and baffle.

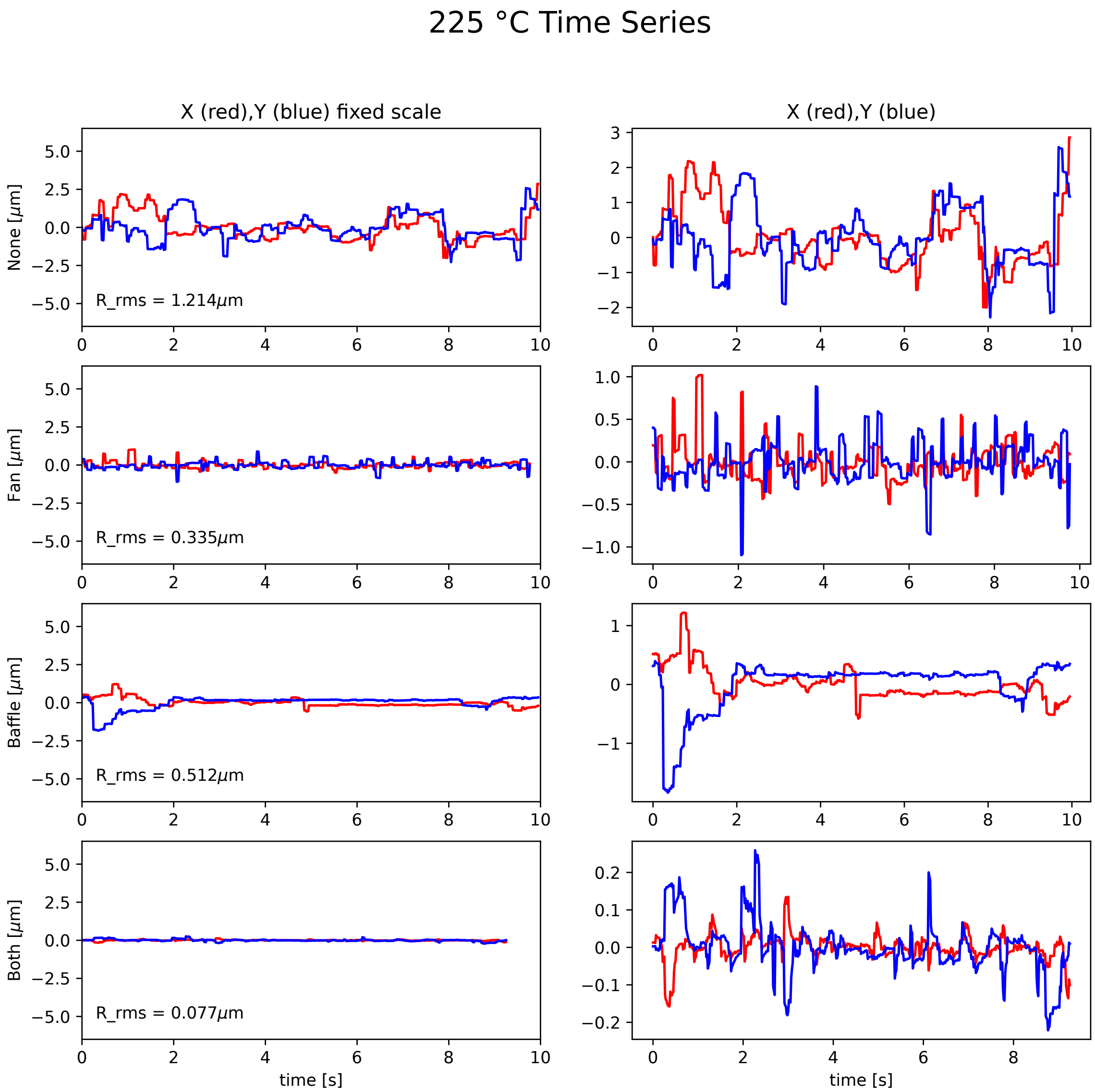

FIG. 8. Time series data of displacement of the tracked point at 225 °C. Note the two columns show identical data–the left side plotted with a fixed vertical scale. From top to bottom, the panels show no intervention, fan only, baffle only, and both fan and baffle.

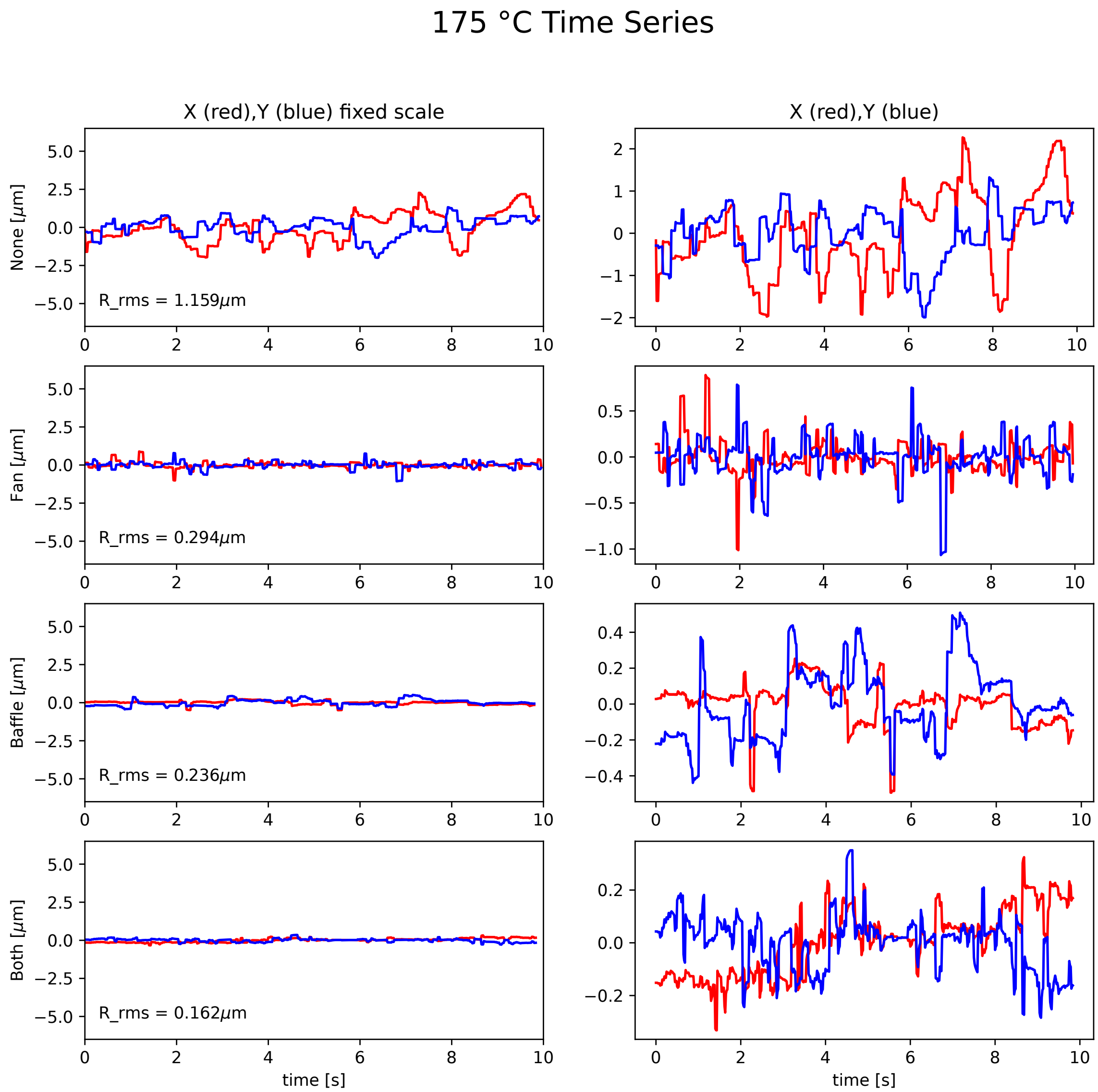

FIG. 9. Time series data of displacement of the tracked point at 175 °C. Note the two columns show identical data–the left side plotted with a fixed vertical scale. From top to bottom, the panels show no intervention, fan only, baffle only, and both fan and baffle.

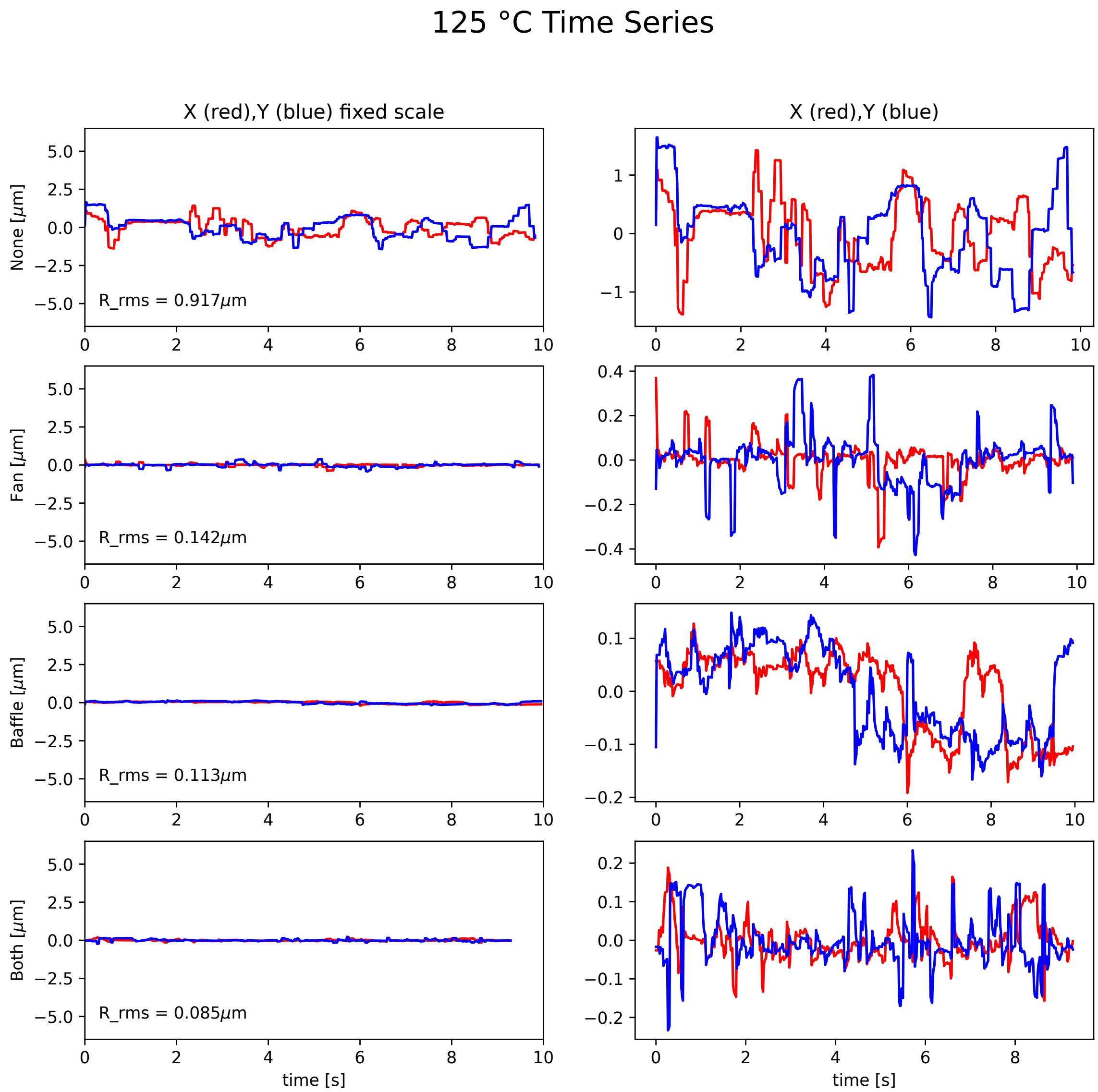

FIG. 10. Time series data of displacement of the tracked point at 125 °C. Note the two columns show identical data–the left side plotted with a fixed vertical scale. From top to bottom, the panels show no intervention, fan only, baffle only, and both fan and baffle.

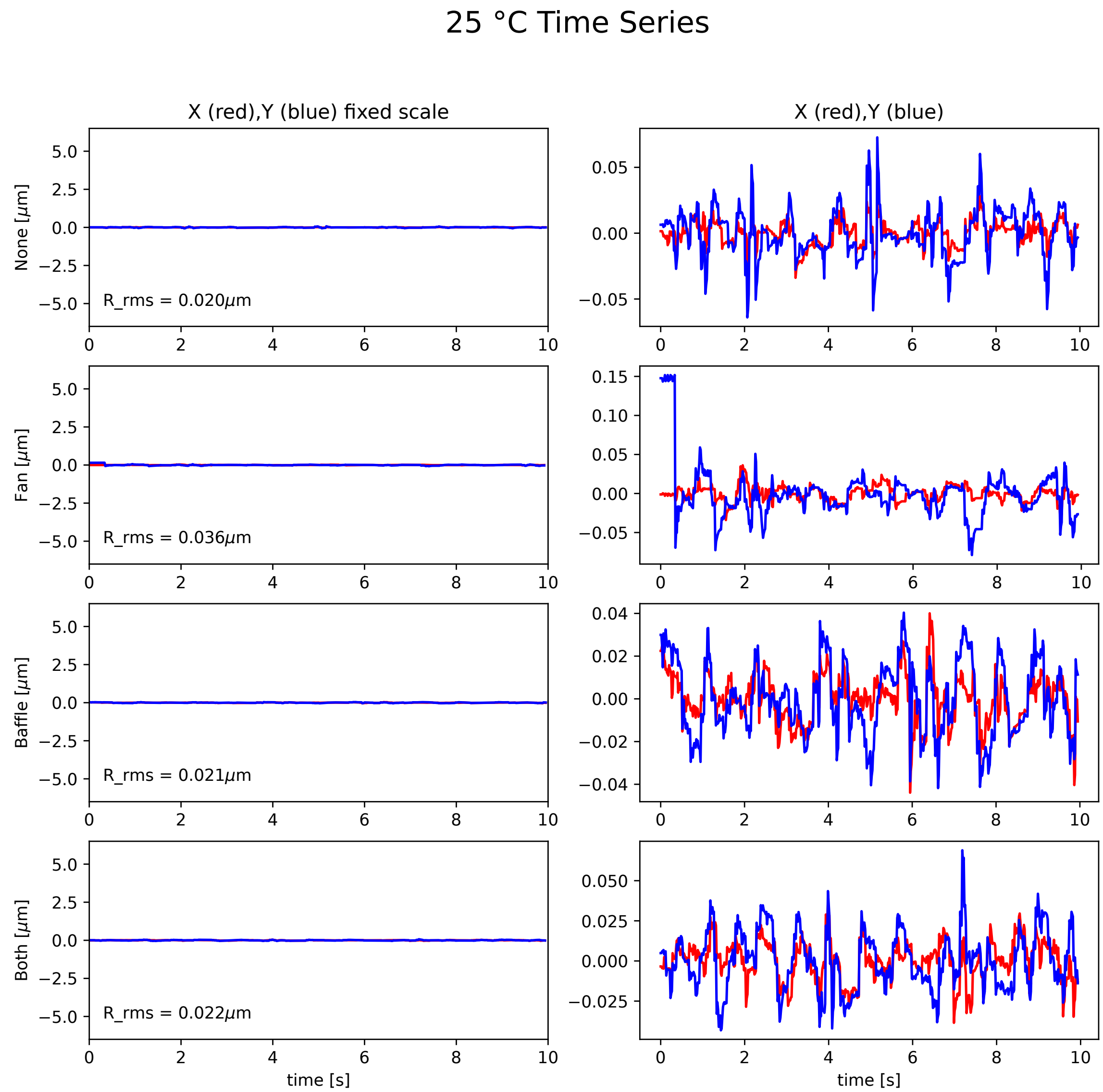

FIG. 11. Time series data of displacement of the tracked point at 25 °C. Note the two columns show identical data–the left side plotted with a fixed vertical scale. From top to bottom, the panels show no intervention, fan only, baffle only, and both fan and baffle.