Dynamic window sizes for contrast estimation in interferometric scattering

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Abstract—Interferometric scattering microscopy is a label-free technique that can detect and track nanoscale matter such as individual proteins. Recently, this technique has been adapted to estimate the mass of proteins due to recent hardware and software advancements. In this work, we attempt to use more sophisiticated methods to more accurately estimate the scattering contrast of the particule using dynamic window sizes calculated via changepoint detection methods.

1 Introduction

Interferometric scattering microscopy is a label-free technique that can detect and track nanoscale matter such as individual proteins [1]. In an interferometric detection scheme, the scattering signal scales with the polarizability, which is a function of the refractive index and proportional to the particle volume [2]. The signal of interest is accompanied by an imaging background which oftentimes is that of a random speckle-like pattern.

There are various sources of noise in an interferometric detection scheme. These include but are not limited to laser intensity noise, detector background noise, and mechanical instability [3]. Recent advancements in the experimental setup and data processing have enabled the ability to extract the scattering contrast for indvidual proteins diffusing in solution that bind nonspecifically to the microscope coverslip [2]. The scattering contrast is then mapped to a molecular weight via mass calibration. In order to extract an accurate estimate of the scattering contrast, the background signal has to be removed after a binding event is detected (Fig. 1). The resulting point-spread function (PSF) in interferometric scattering resembles a concentric ring structure that arises from the interference of plane and spherical waves [3].

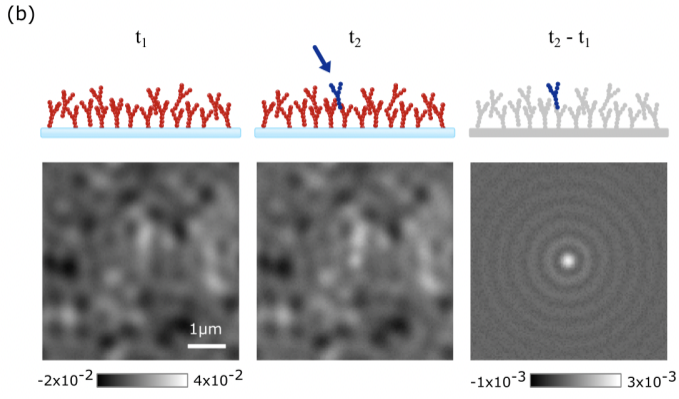

Fig. 1. Background subtraction through differential imaging. To extract the scattering contrast of the binding event, one can theoretically subtract the image of the substrate at a time before t_1 arrival, and one after t_2. The difference isolates the presence of the target protein. Figure reproduced from [3].

2 Related Work

In order to extract the scattering contrast, one needs to detect the occurrence of a binding event and then remove the background. Young et. al accomplished this through a ratiometric imaging approach [2]. Specifically, for each frame, they computed the average of w consecutive frames, resulting in an averaged image $I_{i:i+w-1}$. By computing this averaged image for all frames, one can construct a ratiometric image stack R_w of window size w, where each image $R_{w,i}$ corresponds to $I_{i:i+w-1}/I_{i+w:i+2w-1} - 1$. (Note, different authors use different conventions for the contrast, but to facilitate a comparison to the benchmark method, negative contrast corresponds to a binding event). By constructing this ratiometric image stack, one can detect the occurrence of a binding event. Specifically, when $I_{i+w:i+2w-1}$ only consists of frames after the binding event occurred, the absolute value of the scattering contrast at the center of the PSF will be maximized (Fig. 2). Upon detection of a binding event, an N*N region centered at the candidate pixel of interest is extracted and then fit to a model PSF to extract the contrast.

Because inteferometric scattering is a shot-noise limited process, averaging images within a window w results in a more accurate estimate of the scattering contrast. Given this, one may naturally wonder why one cannot indefinitely extend the window size? Young et. al investigated the effect of increasing the size, but observed that past a certain window size, the noise actually increases [2]. This is likely due to sample drift and the fluctuating nature of the background noise. In other words, the background signal and particle signal vary as a function of time, so averaging with larger window sizes effectively convolutes the actual PSF.

3 Methods

The primary aim of this work was to investigate if using a more sophisticated background detection method can lead to an improved estimate of the scattering contrast. To do so, we employed a statistical method known as changepoint detection to find an optimal window size in both the forward and backwards direction upon detection of a binding event. The basic idea behind changepoint detection is to

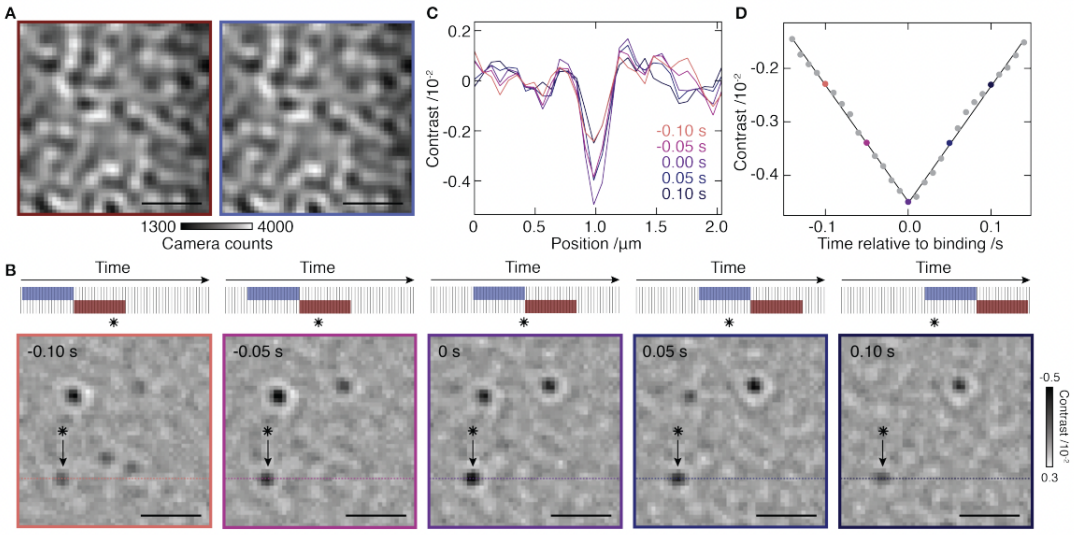

Fig. 2. (A) Raw camera images before and after the landing event in B-D showing image contrast due to coverslip roughness. (B) Illustration of the image averaging and differential imaging approach. The asterisk marks a landing event. Individual images are averaged into two consecutive blocks (blue and red), which are divided to provide differential contrast. The mid-point is scanned in time, meaning that the signal from stochastic landing events grows and fades, as indicated by the black arrow. Scale bars: 1 μm. (C) Corresponding cross-sections for the particle highlighted in B. (D) Corresponding signal magnitudes extracted by a fit to the PSF and fit (black). Figure reproduced from [2].

identify times when the probability distribution of a stochastic process or time series changes. We experimented with both frequentist and bayesian methods [4,5]. The frequentist method outputs a single index corresponding to the frame of the changepoint while the bayesian method outputs a number between 0 and 1 for each frame corresponding to its probability being a changepoint. Assuming that a binding event occurs at frame f centered at pixel j, k, applying a changepoint detection algorithm in the forwards and backwards direction will yield frames f_f and f_b corresponding to the frames at which the distribution of the particle and background signal changes. Presumably, this would lead to a more robust estimation of the scattering contrast assuming that the fluctuation of the background and particle signal is itself stochastic. This is likely a reasonable assumption given that sample drift is a diffusion based process that can be modeled by brownian motion.

3.1 Quantifying the noise of a background signal with and without changepoint detection

To assess the efficacy of dynamic window approach, we first quantified the noise of the signal in the absence of any protein (i.e buffer only). To quantify the noise for a ratiometric image stack of background signal, we calculated the standard deviation of $R(j,k)$ for pixels j, k that were uniformly spaced in the image. For a fixed window size, this corresponds to calculating the standard deviation of $R_w(j,k)$. This was done for window sizes ranging from 1 to 40. For reference, the frame rate in our experiments was 50 Hz, so a window size of 40 frames corresponds to an integration time of .8 seconds.

To construct the ratiometric image stack with dynamic window sizes for background signal, we applied the following procedure: 1) For a search window size w, apply a changepoint detection algorithm between $I_{i:i+w-1}(j,k)$ to calculate the frame f_c indicating where the distribution of $I_{i:i+w-1}(j,k)$ changes. 2) Let f_m refer to $f_c/2$. $R_{w,i}$ then corresponds to $I_{i:i+f_m-1}/I_{i+f_m:i+f_c-1} - 1$. 3) Let $i = f_c$ and repeat this procedure while $i < f_l$ where f_l is the last frame of the image data. For the frequentist changepoint detection method, we experiment with various search window sizes. For the bayesian changepoint detection method, we used a fixed search window size of 100, but experimented with various probability thresholds to classify whether or not a frame corresponds to a changepoint. The pseudo-code is summarized below:

$i \leftarrow 0$
while $i < f_l$ **do**
  $f_c \leftarrow cp(I_{i:i+w-1}(j,k))$
  $f_m \leftarrow f_c/2$
  $R_{w,i} \leftarrow I_{i:i+f_m-1}/I_{i+f_m:i+f_c-1} - 1$
  $i = f_c$
end while

3.2 Calculating ratiometric images for binding events with dynamic window sizes

Assume a particle event is detected at frame i for pixels j, k. (Note, the proprietary software coupled to the interferometic scattering instrument outputs the location and frame at which an event is detected. We use this information to facilitate an accurate comparison to the software's results. In theory, the changepoint detection algorithm itself can be used to both detect the event and calculate the optimal window sizes).

To construct the ratiometric image stack with dynamic window sizes for the particle signal, we applied the following procedure for each binding event detected by the

software: 1) For a search window size w, apply a changepoint detection algorithm in the forward direction between $I_{i:i+w-1}(j,k)$ to calculate the frame f_f indicating where the distribution of $I_{i:i+w-1}(j,k)$ changes. For a search window size w, apply a changepoint detection algorithm in the backward direction between $I_{i-w:i-1}(j,k)$ to calculate the frame f_b indicating where the distribution of $I_{i-w:i-1}(j,k)$ changes. 2) $R_{w,i}$ then corresponds to $I_{i:f_f-1}/I_{f_b:i-1}-1$. We only implemented this method for the bayesian changepoint detection method, and used a window size of 100 and a probability threshold of .15. The pseudo-code is summarized below:

$i \leftarrow 0$
$N \leftarrow num\_events$
while $i < N$ **do**
  $f_f \leftarrow cp(I_{i:i+w-1}(j,k))$
  $f_b \leftarrow cp(I_{i-w:i-1}(j,k))$
  $R_{w,i} \leftarrow I_{i:f_f-1}/I_{f_b:i-1}-1$
  $i = i+1$
end while

3.3 Calculating the scattering contrast

Assume a particle event is detected at frame i for pixels j,k. We calculated the ratiometric image stack via changepoint detection for $I_i(j-3:j+3,k-3:k+3)$. This yields $R_i(j-3:j+3,k-3:k+3)$. To extract the final scattering contrast, Young et. al fit the data to a model PSF which was of the form of the difference of two concentric 2D Gaussians [2]. However, subsequent publications use a different PSF, and the precise PSF used in the software is unknown. Instead of using a model PSF, a Gaussian Process Regression (GPR) model with a RBF kernel was used to fit the data. This GPR model was then predicted for $R_i(j-1:j+1,k-1:k+1)$ at uniformly spaced intervals of .2, yielding up to 121 predictions. (Note: even though the data is discretely sampled, the GPR allows us to make predictions in a continuous domain. For reference, the software coupled to the instrument also outputs floating point values for the location of the binding events). The final contrast is then the minimum of all predictions between $j-1:j+1,k-1:k+1$. Because the data is sparsely sampled and noisy, fitting the data to a function first effectively acts as a denoising step. The pseudo-code is summarized below. Note that GPR_{fit} is a function while GPR_{pred} is a vector.

$GPR_{fit} \leftarrow GPR(R_i(j-3:j+3,k-3:k+3))$
$GPR_{pred}(:) \leftarrow GPR_{fit}(R_i(j-1:j+1,k-1:k+1))$
$contrast = min(GPR_{pred}(:))$

4 Experimental Results

4.1 Noise of a background signal with and without changepoint detection

In Fig. 3 and 4, we illustrate the results of comparing the standard deviation of $R(j,k)$ for pixels j,k that were uniformly spaced in the image. This was done for the fixed and dynamic window sizes for both the frequentist (Fig. 3) and bayesian approach (Fig. 4). As can be seen, when using a fixed sized window, the standard deviation of $R(j,k)$ increases past a certain window length. However, when using a dynamic window size based on a change point detection method, $R(j,k)$ remains roughly constant and consistently lower than its fixed window size counterparts. Note that for the dynamic window size approach, we naturally calculate $R(j,k)$ for a variety of different window sizes, enabling a direct comparison of the standard deviation for the same number of data points.

4.2 Illustrative example of bayesian changepoint detection

We illustrate the potential benefit of using a bayesian changepoint detection method for a given binding event in Fig. 5. As one can see, in the forward direction the distribution of the signal remains roughly constant for 45 frames while in the backward direction the distribution of the signal remains roughly constant for only 10 frames. By picking the window size in a data dependent manner, it stands to reason we may more accurately estimate the scattering contrast. In Fig. 6, we quantified the distribution of frames used in the forwards and backwards direction.

4.3 Scattering contrast comparison

As described in the methods section, we calculated a ratiometric image stack for each binding event using dynamic window sizes and then extracted the scattering contrast using a GPR model. This enabled us to compare the contrasts outputted by the software coupled to the instrument. In Fig. 7, we evaluate the pearson correlation between the two methods, and we overall see a good fit (R = .99). In Fig. 8, we display a comparison of the kernel density estimates for the protein bovine serum albumin (BSA). This protein exists as both a monomer and dimer, so two distinct peaks are seen in the plots. Note that contrasts greater than 0 are observed, and these correspond to unbinding events, which are not physically relevant in the context of mass estimation. Overall, the peaks overlap quite well, though we do observe that for the dimeric species, the width of the peak is noticeably larger for the dynamic window method compared to the benchmark. In Fig. 9, we quantitatively evaluate the resolution by comparing the full-width at half maximum (FWHM) for each population for each of the methods. While our methodology did not improve the contrast resolution, we do note that we did not use the same (unknown) PSF fitting procedure as the benchmark. We speculate that using a more sophisticated fitting procedure may act as a more effective denoiser by incorporating information regarding the theoretical PSF. In support of this idea, we display the results of our method without using a GPR regression model to calculate the scattering contrast (Fig. 10). Rather, we simply use the minimum scattering contrast in the corresponding pixel neighborhood. We observe markedly worse contrast resolution, highlighting the effectiveness of the fitting procedure.

5 Conclusion

Using dynamic window sizes when calculating ratiometric image stacks may serve as a promising alternative to using fixed window sizes. The fact that the background noise level is reduced when comparing window sizes of the same length is indicative of this. Though this did not immediately

translate to improved contrast resolution, more sophisticated procedures for fitting a model PSF to the contrast data may lead to further improvements and a more standardized comparison between both methods.

Acknowledgments

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Code and Data

https://drive.google.com/file/d/1O-LKnnGZpP_BZqFNRLQdBKuLcIaupuRh/view?usp=sharing

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3. Richard W. Taylor and Vahid Sandoghdar. 2018. "Interferometric Scattering (iSCAT) Microscopy Related Techniques." *arXiv*. http://arxiv.org/abs/1812.10765.

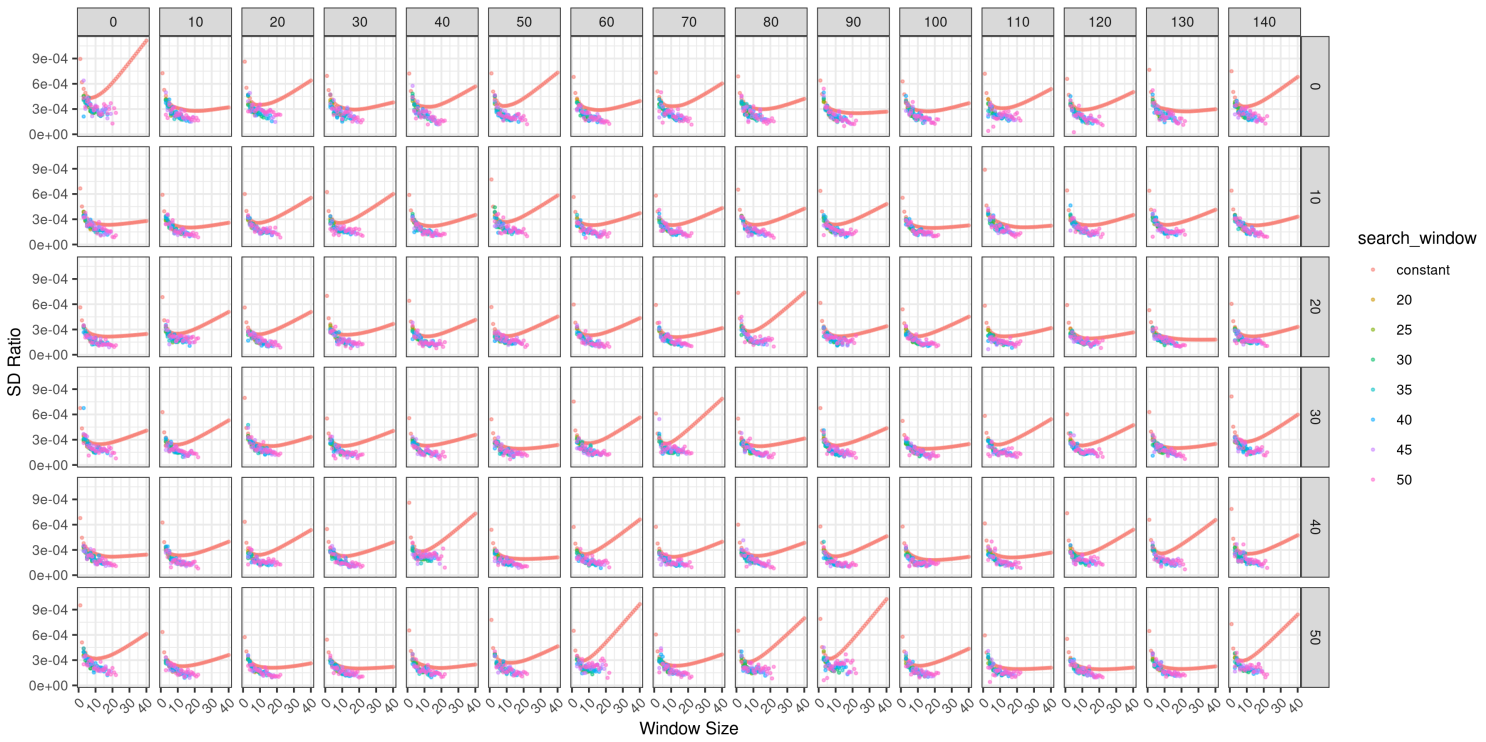

Fig. 3. Comparison between $R(j,k)$ for a fixed window size (red) and dynamic window size using a frequentist changepoint detection method. For this method, we experimented with search window sizes ranging from 20 to 50 in increments of 5. Each column corresponds to an x coordinate in the image while each row corresponds to a y coordinate in the image

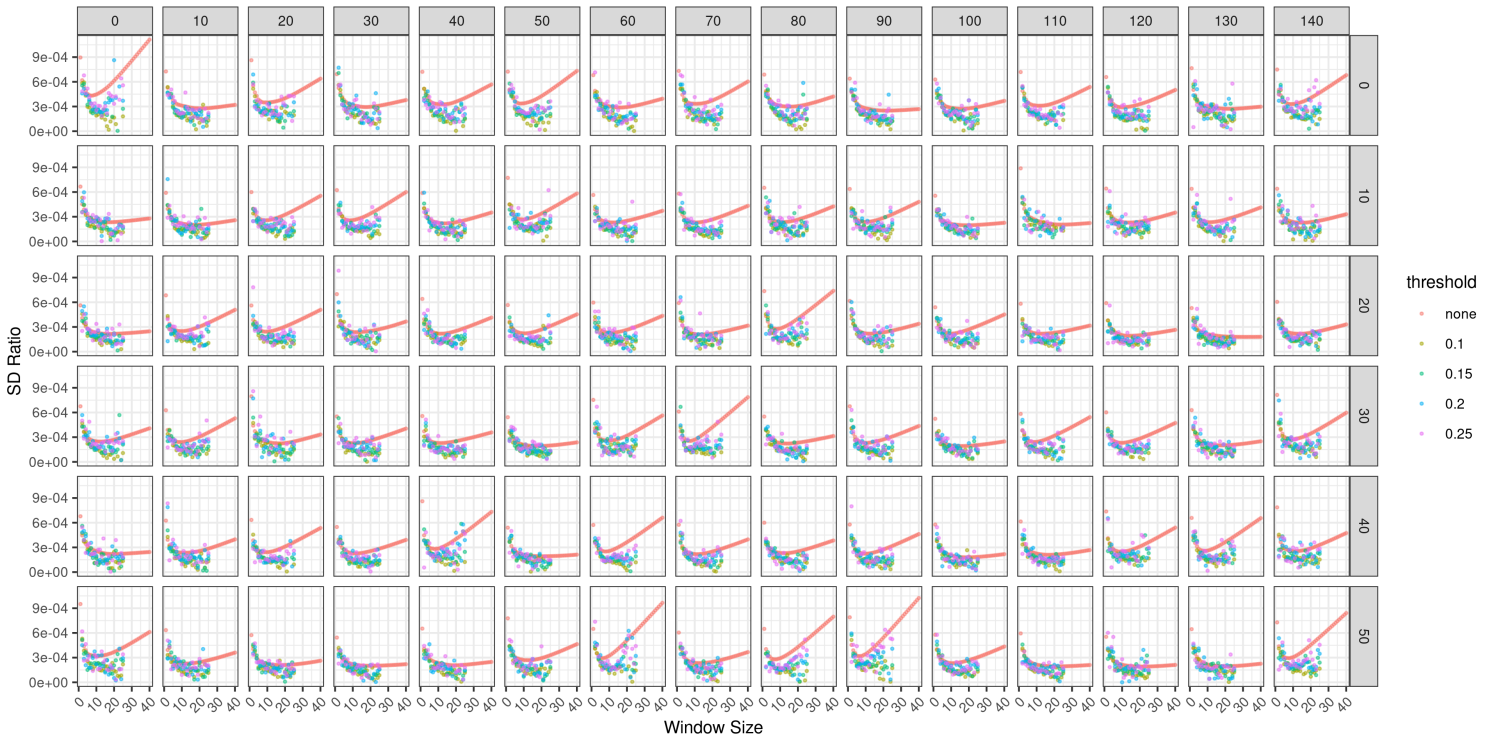

Fig. 4. Comparison between $R(j,k)$ for a fixed window size (red) and dynamic window size using a bayesian changepoint detection method. For this method, we experimented with probability thresholds ranging from .1 to .25 in increments of .05. Each column corresponds to an x coordinate in the image while each row corresponds to a y coordinate in the image

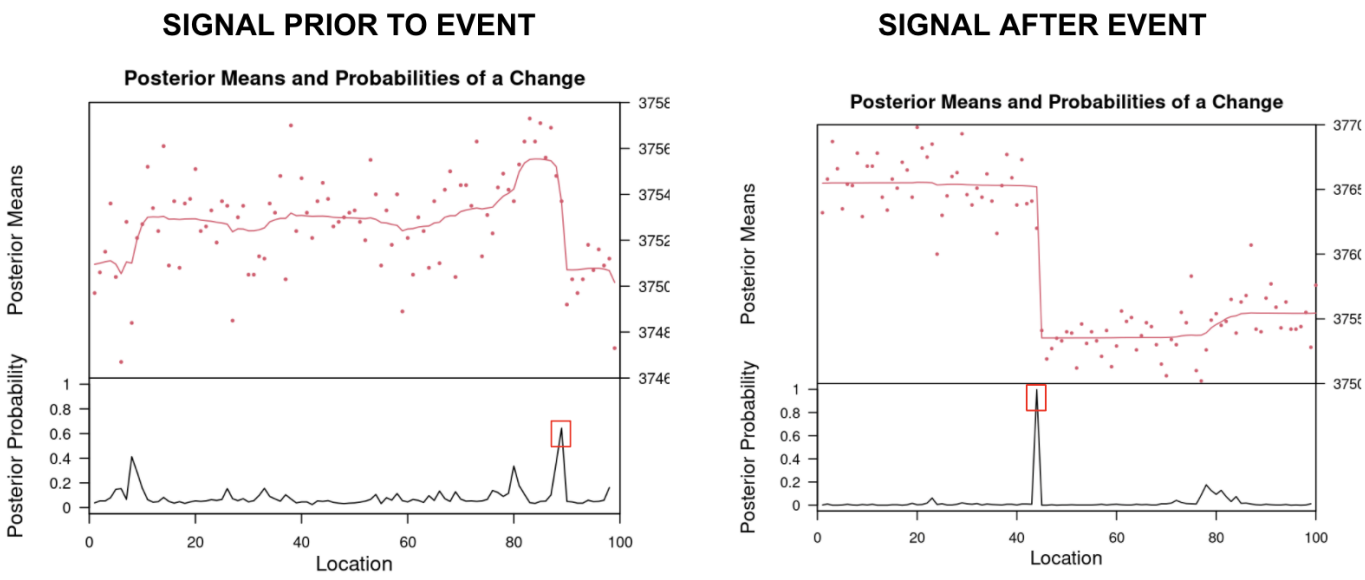

Fig. 5. Bayesian changepoint detection applied to a binding event within a window of 100 frames forward and backwards. The red square corresponds to either the first or last frame used to calculate the mean signal.

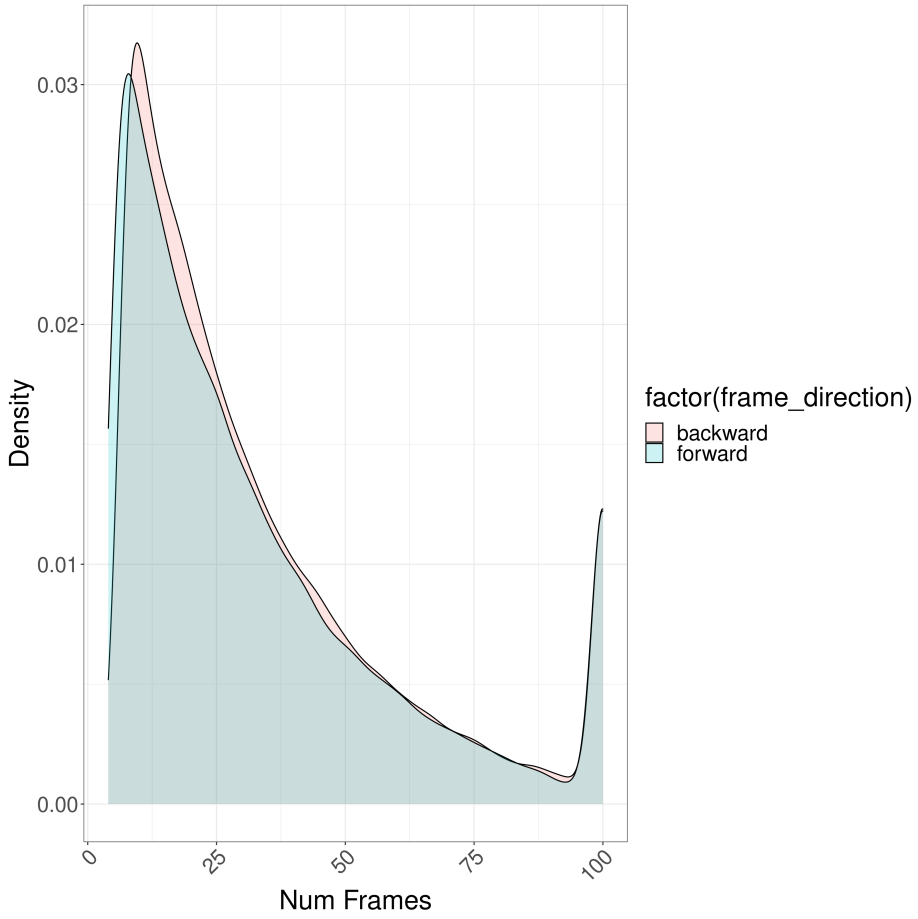

Fig. 6. Distribution of the number of frames used in the forwards and backwards direction using the bayesian changepoint detection method.

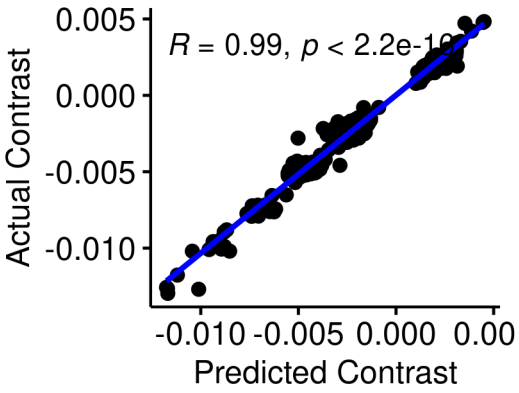

Fig. 7. Scatter plot comparing the predicted contrast to the actual contrast outputted by the software.

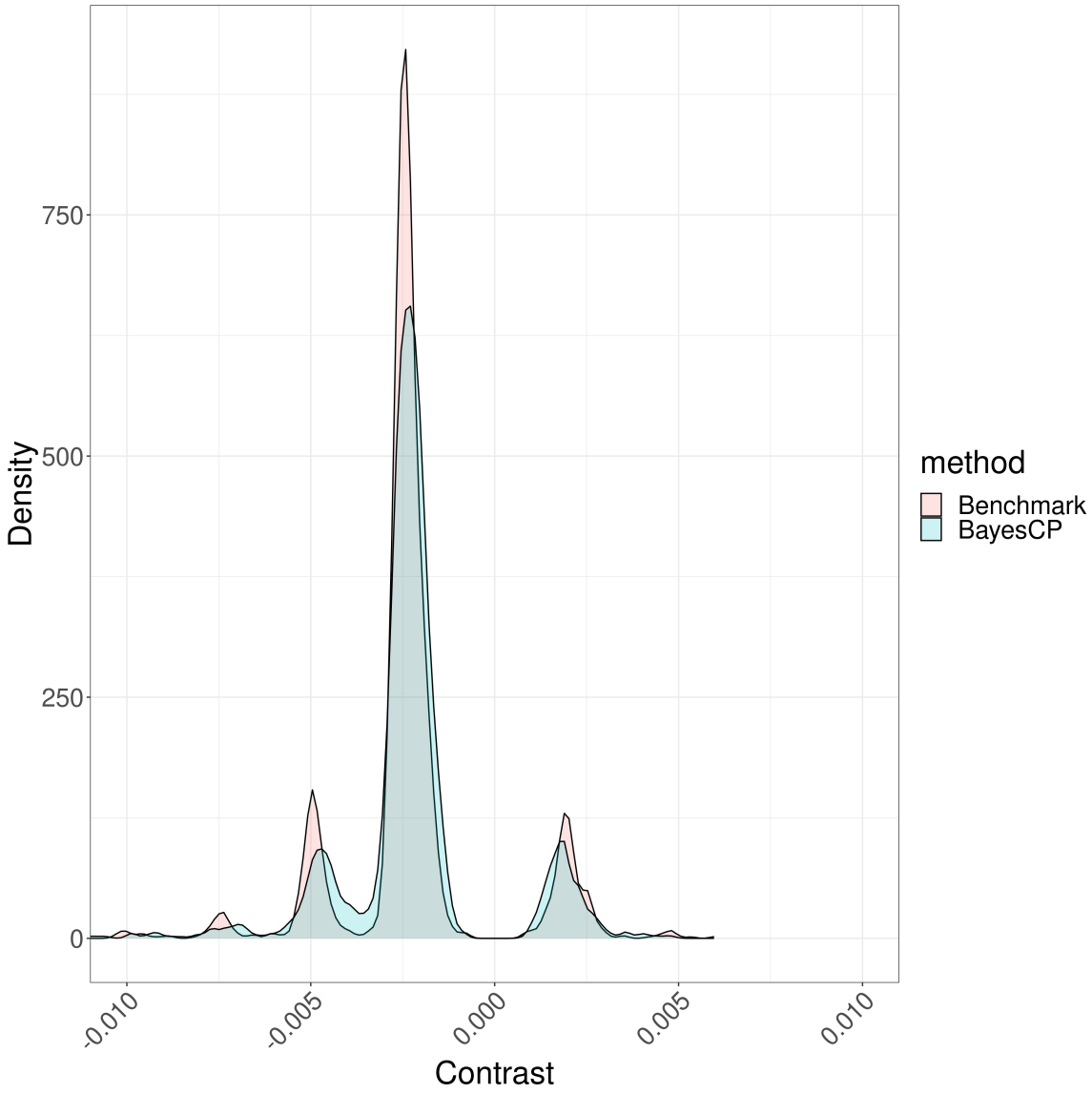

Fig. 8. Kernel density plot comparing predicted contrast to actual contrast outputted by the software.

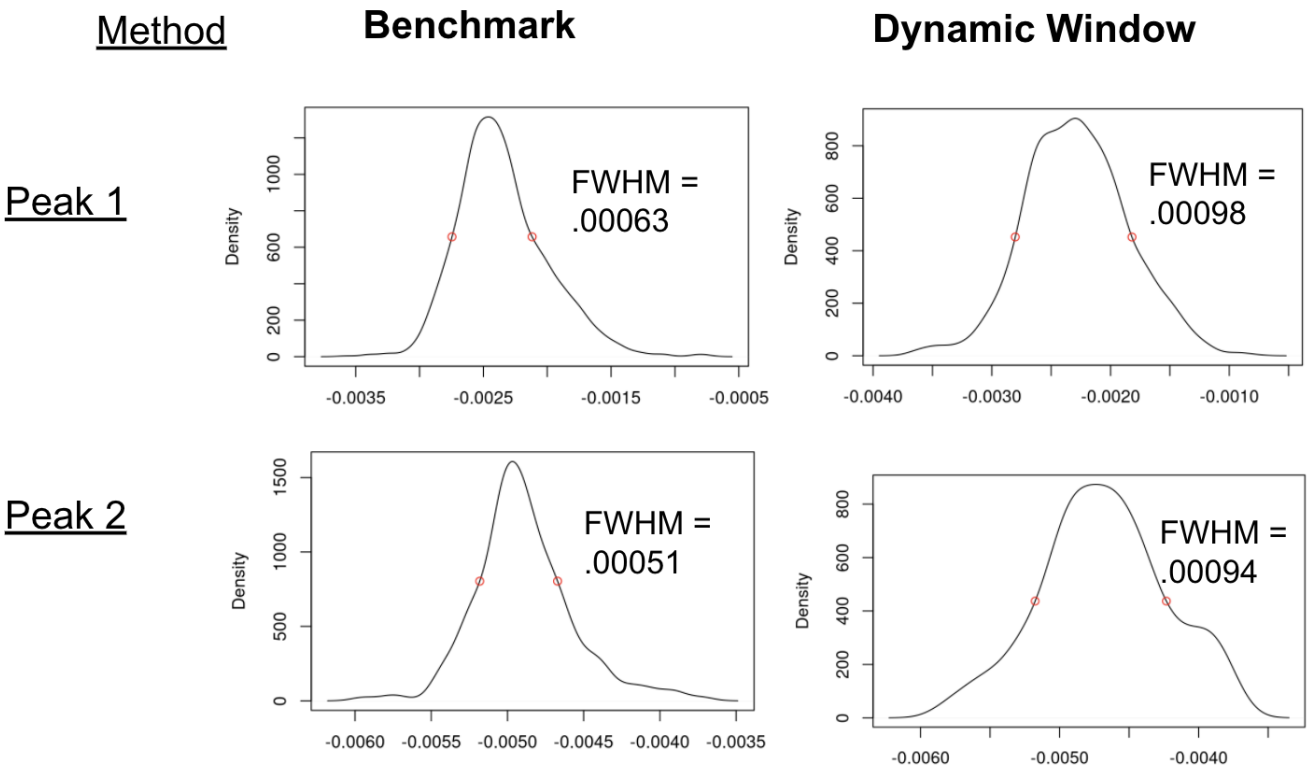

Fig. 9. Comparison of the FWHM for each population for each method.

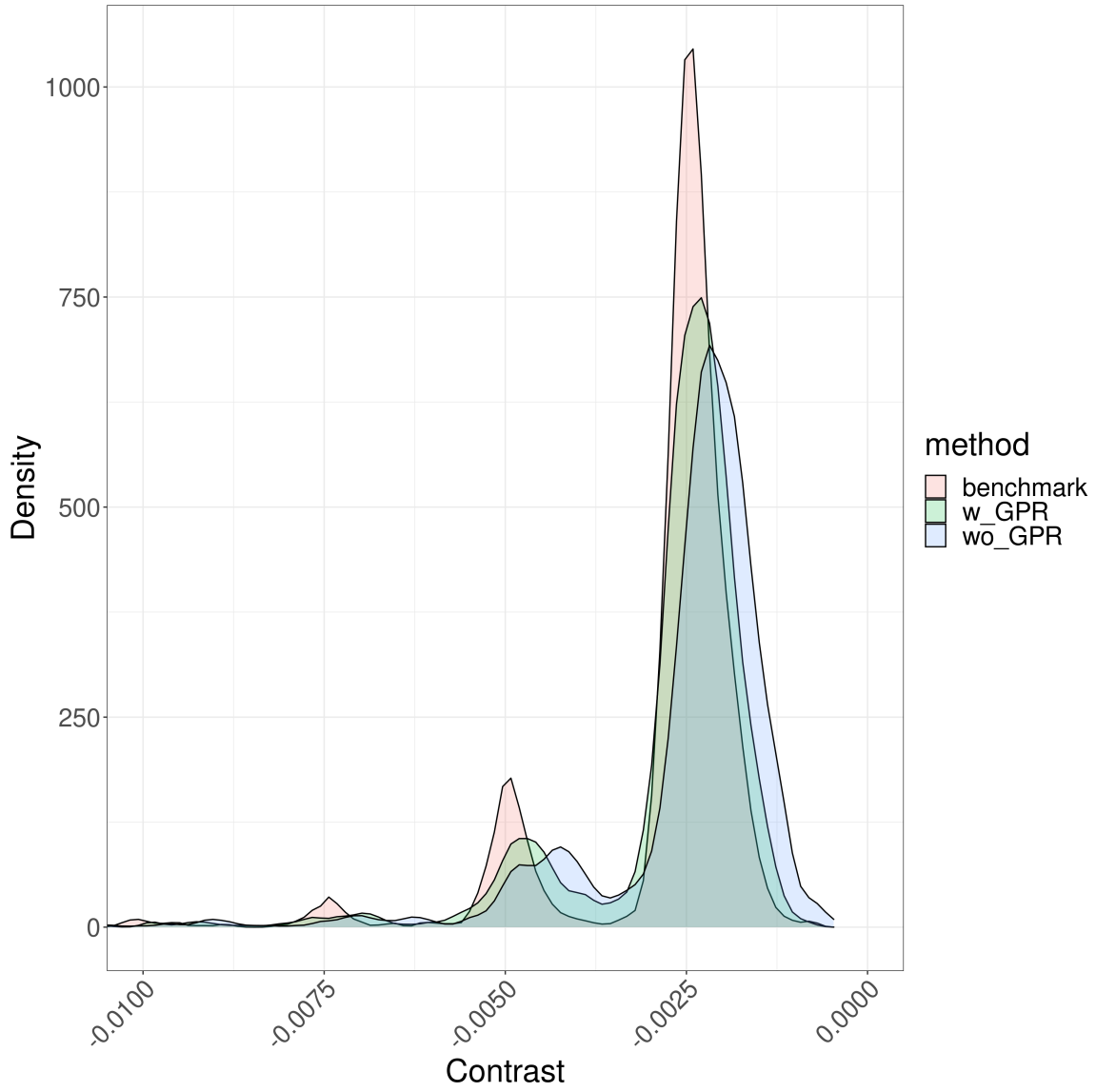

Fig. 10. Kernel density plot comparing the predicted contrast with and without using the GPR fitting procedure to the actual contrast outputted by the software .