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Data Article

Time-shifted mean-segmented Q data of a luminal protein measured at the nuclear envelope by fluorescence fluctuation microscopy

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ABSTRACT

Fluorescence fluctuation microscopy is a widely used method to determine the mobility and oligomeric state of proteins in the live cell environment. Existing analysis methods rely on statistical evaluation of data segments with the implicit assumption that no significant signal fluctuations occur on the time scale of a data segment. Recent work on extending fluorescence fluctuation methods to the nuclear envelope of living cells identified a slow fluctuation process that is associated with the undulations of the nuclear membranes, which lead to intensity fluctuations due to local volume changes at the nuclear envelope. This environment violates the above-mentioned assumption and is associated with biased evaluation of fluorescence fluctuation data by traditional analysis methods, such as the autocorrelation function. This challenge was overcome by the introduction of the time-shifted mean-segmented *Q* function, which relies on a sliding scale of data segment lengths. Here, we share experimental fluorescence fluctuation data taken at the nuclear envelope and demonstrate the calculation of the time-shifted mean-segmented *Q* function from the raw data. The data and analysis should be valuable for researchers interested in fluorescence fluctuation techniques and provides an opportunity to examine the influence of slow fluctuations on existing data analysis methods. The data is related to the research article titled "Protein oligomerization and mobility within

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the nuclear envelope evaluated by the time-shifted mean-segmented Q factor" [1].

Specifications Table

Subject	Biophysics
Specific subject area	Fluorescence fluctuation spectroscopy
Type of data	Photon count data of fluorescent proteins at the nuclear envelope
How data were acquired	Custom optical two-photon microscope
Data format	Raw and graph
Parameters for data collection	U2OS cells were transiently transfected with plasmid leading to the targeting of EGFP to the lumen of the nuclear envelope. Cells on glass slide were mounted on microscope stage for fluorescence fluctuation measurement
Description of data collection	Two-photon spot was focused on the nuclear envelope of transiently transfected cells to excite luminal EGFP. Photon count data were collected for ~60 s with a sampling frequency of 20 kHz.
Data source location	Institution: University of Minnesota, Twin Cities City/Town/Region: Minneapolis, Minnesota Country: USA
Data accessibility	Repository name: Mendeley Data https://doi.org/10.17632/jcknwhy4r8.2
Related research article	J. Hennen, K.H. Hur, S.R. Karuka, G.W.G. Luxton, J.D. Mueller, Protein oligomerization and mobility within the nuclear envelope evaluated by the time-shifted mean-segmented Q factor, Methods. 157 (2019) 28–41. https://doi.org/10.1016/j.ymeth.2018.09.008.

Value of the Data

- The data presented here are acquired at the nuclear envelope of a living U2OS cell and contain the fluorescence intensity fluctuations from luminal EGFP. The data are a useful resource to examine the fluctuations in this environment both with existing analysis techniques, such as the autocorrelation function, and with newer techniques like the time-shifted mean-segmented Q (*tsMSQ*) analysis.
- The data in this article can serve as a valuable reference for investigators interested in applying fluorescence fluctuation methods at the nuclear envelope.
- The shared data are likely to be valuable in further development and refinement of unbiased fluorescence fluctuation algorithms by providing a benchmark dataset for quantitative evaluation of analysis results with *tsMSQ*.
- The data contain diffusion fluctuations of luminal EGFP and a slow fluctuation process due to the local volume changes at the nuclear envelope. The data can be of additional value to investigators studying fluctuations arising from two processes with distinct temporal time scales.

1. Data description

The data presented here are experimental recordings of the fluorescence intensity of EGFP targeted to the lumen of the nuclear envelope (NE) of a U2OS cell (Fig. 1A). A conceptual picture of the NE illustrates the ~40 nm-wide lumen containing EGFP proteins sandwiched between two nuclear membranes (Fig. 1B). Photon count data (Fig. 2A) were collected with the two-photon excitation spot centered on the NE (Fig. 1A). While the conceptual picture suggests that the fluctuations in the fluorescence intensity (Fig. 1C) solely reflect the diffusion of EGFP across the two-photon spot, it was recently found [2] that the distance between the two nuclear membranes undulates (Fig. 1D), leading to local volume fluctuations, which superimpose a slow fluctuation process on top of the diffusional component, as conceptually illustrated in Fig. 1E.

The raw data (Fig. 2A) were analyzed using time-shifted mean-segmented Q (*tsMSQ*) analysis (Fig. 2B), a recently developed method, to avoid the biases introduced by the slow undulation process

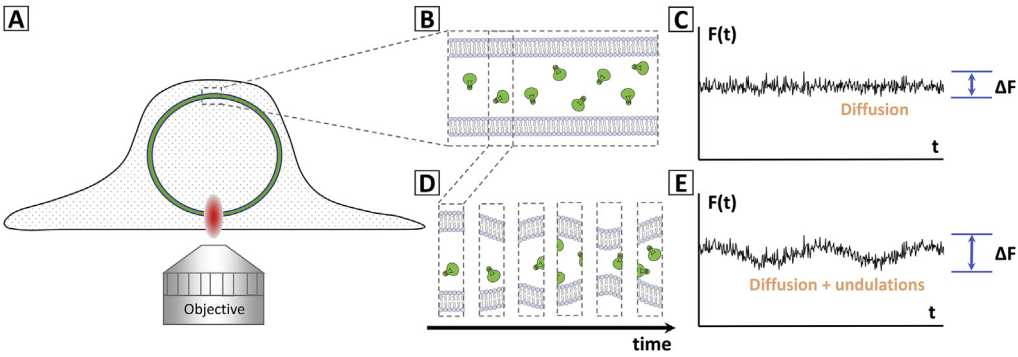

Fig. 1. Illustration of data collection process at the NE of a living cell. (A) The two-photon excitation spot is focused at the ventral NE. (B) Conceptual picture of the NE with two nuclear membranes and luminal EGFP (green lightbulbs). (C) Illustration of fluorescence fluctuation data expected from pure diffusion of luminal EGFP. (D) Shown in this conceptual picture is a small segment of NE with temporal undulations in the distance separating both membranes. (E) Shown here is a conceptual illustration of fluorescence fluctuation data that include the additional undulation process.

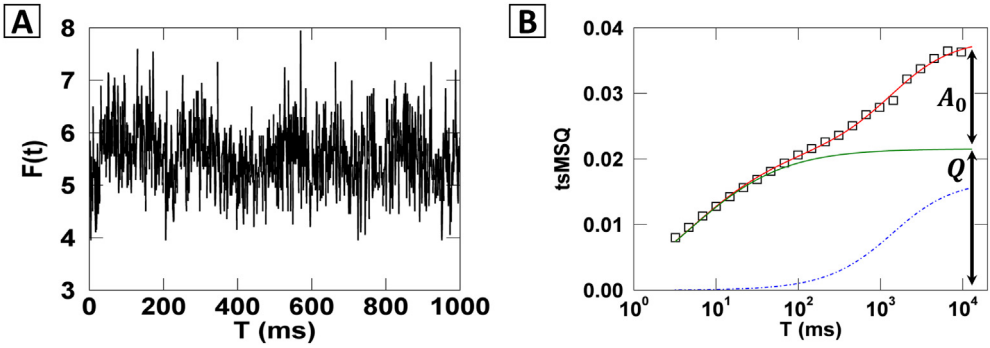

Fig. 2. *tsMSQ* of photon count data. (A) Photon count data of luminal EGFP were taken for 60 s. A 1 s segment of the data set is shown with photon counts averaged over 1 ms. (B) *tsMSQ* (squares) versus segment time T of the full 60 s photon count data from panel A. Solid red line is a fit to a diffusion model that includes NE undulations. Solid green line is the diffusion component and the dashed blue line represents the NE undulation component.

into conventional autocorrelation analysis of the data [1]. The *tsMSQ* curve determines the diffusion time τ_D and diffusion amplitude Q of luminal EGFP as well as the characteristic time τ_0 and amplitude A_0 of the undulation process (Fig. 2B). The unbiased separation of local volume fluctuations from diffusional fluctuations achieved by *tsMSQ* was a prerequisite for successful investigation of protein assembly at the NE by fluorescence fluctuation spectroscopy [1].

2. Experimental design, materials, and methods

Extensive details of our experimental setup and data collection process have been described elsewhere [3]. Briefly, a home-built two-photon microscope excites the sample at a wavelength of ~1000 nm at sufficiently low power (0.2–0.4 mW) to avoid photobleaching artifacts. A C-Apochromat water immersion objective with NA = 1.2 (Carl Zeiss AG, Jena, Germany) focuses the excitation light onto the sample and collects the emitted photons, which are detected by a single-photon counting module (SPCM-AQR-14, PerkinElmer, Dumberry, Quebec) and recorded by a Flex04-12D (correlator.com, Bridgewater, NJ) data acquisition card for subsequent analysis.

The U2OS cells (ATCC, Manassas, VA), maintained in DMEM with 10% FBS (Hyclone Laboratories, Logan, UT), were plated in a 24-well glass-bottom slide with #1.5H coverglass (In Vitro Scientific,

Sunnyvale, CA) approximately 48 hours before measurement. Cells were transiently transfected with GenJet (SignaGen Laboratories, Rockville, MD) 24 hours prior to measurement, according to the manufacturer's instructions. A plasmid containing EGFP fused with the signal sequence of the luminal protein torsinA was used to ensure efficient localization of EGFP to the shared lumen of the endoplasmic reticulum and the NE [4,5]. On the day of measurement, the medium was replaced with DPBS premixed with calcium and magnesium (130 mg/L calcium chloride dihydrate and 100 mg/L magnesium chloride hexahydrate) by the manufacturer (BioWhittaker, Walkerville, MD) followed by mounting of the 24-well glass-bottom slide onto the stage of the microscope.

A cell expressing luminal EGFP is identified by brief epifluorescence exposure of the sample. After centering the selected cell with the stage, the axial localization of the fluorophore is checked by scanning the two-photon excitation spot along the optical axis as previously described [6]. Next, the two-photon excitation spot is focused on the ventral NE and data is collected for ~60 seconds. The same procedure is then repeated with the focus shifted to the dorsal NE, before moving on to the next cell. The recorded photon data were analyzed with the *tsMSQ* algorithm implemented in IDL.

The algorithm used to construct the *tsMSQ* curve from experimental photon count data is depicted in Fig. 3 using a very short photon count record for illustrative purposes. The steps for calculating the *tsMSQ* value for segment time T_x are as follows:

1. The photon counts k recorded with a sampling time T_s are divided into segments of length T_x (Fig. 3A).
2. The time-shifted Q value (tsQ) of the segment is calculated using the equation $tsQ = \frac{\langle (k_{i+1} - \langle k_{i+1} \rangle)(k_i - \langle k_i \rangle) \rangle}{\langle k_{i+1} \rangle}$, where k_i and k_{i+1} are the values at the i^{th} and $(i+1)^{th}$ bin of the photon record, respectively. $\langle k_i \rangle$ denotes the average value of the photon record excluding the last bin in the segment. $\langle k_{i+1} \rangle$ denotes the average value of the photon record excluding the first bin in the segment. Pictorially the time shift can be visualized as creating a copy of the photon count data that is shifted by T_s, as shown for the first segment in Fig. 3B. The tsQ is calculated from the red-shaded portion of the segment.
3. The tsQ values of all segments are calculated and their average $\langle tsQ(T_x) \rangle$ is determined (Fig. 3C).

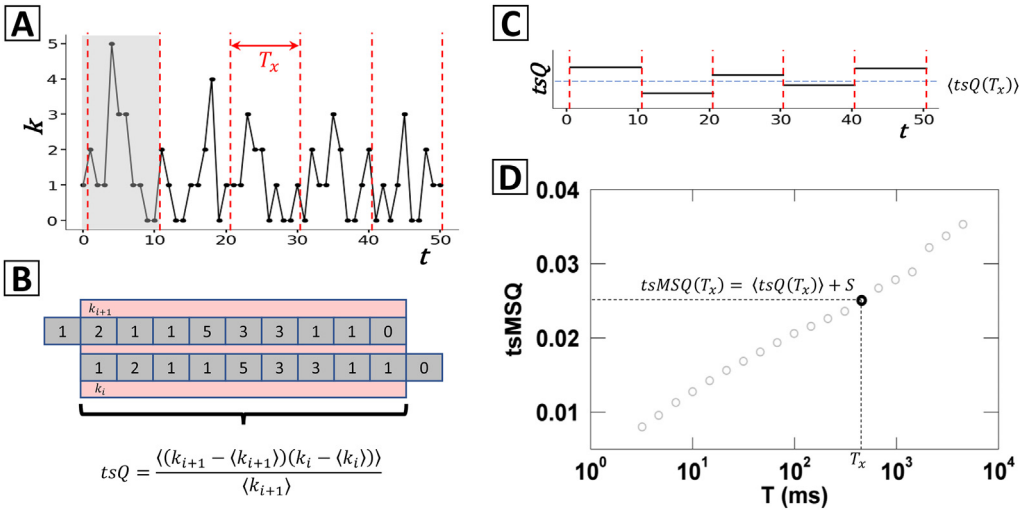

Fig. 3. Illustration of the *tsMSQ* algorithm. (A) Photon count data recorded with sampling time T_s are divided into segments of length T_x. (B) The photon counts of the first segment are shown together with a time-shifted copy, which are used to calculate the tsQ value of the segment. (C) The tsQ values from all segments are averaged (dashed line). (D) The *tsMSQ* value (black circle) for segment time T_x is determined by adding the shot noise term S to the averaged tsQ value. Repeating this process for a range of segment times yields the complete *tsMSQ* curve (gray circles).

4. The *tsMSQ* value for segment time T_x is determined by adding the term $S = \frac{(T_x - 2T_s)T_s}{(T_x - T_s)^2}$ to $\langle tsQ(T_x) \rangle$ (Fig. 3D).

Steps 1 to 4 are repeated over a range of segment times to determine the complete *tsMSQ* curve. Typical segment times range from 10 ms to several seconds (Fig. 3D). The experimental *tsMSQ* curve is fitted to a model accounting for diffusion and NE undulation, as described in detail elsewhere [1]. For the data described in this manuscript the following values were obtained from the *tsMSQ* fit: $A_0 = 0.017$, $\tau_0 = 523$ ms, $Q = 0.022$, $\tau_D = 1.5$ ms.

Acknowledgments

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] J. Hennen, K.H. Hur, S.R. Karuka, G.W.G. Luxton, J.D. Mueller, Protein oligomerization and mobility within the nuclear envelope evaluated by the time-shifted mean-segmented Q factor, Methods 157 (2019) 28–41, https://doi.org/10.1016/j.ymeth.2018.09.008.
[2] J. Hennen, K.H. Hur, C.A. Saunders, G.W.G. Luxton, J.D. Mueller, Quantitative brightness analysis of protein oligomerization in the nuclear envelope, Biophys. J. 113 (2017) 138–147, https://doi.org/10.1016/j.bpj.2017.05.044.
[3] J. Hennen, I. Angert, K.-H. Hur, G.W. Gant Luxton, J.D. Mueller, Investigating LINC complex protein homo-oligomerization in the nuclear envelopes of living cells using fluorescence fluctuation spectroscopy, Methods Mol. Biol. 1840 (2018) 121–135, https://doi.org/10.1007/978-1-4939-8691-0_11.
[4] J. Hennen, C.A. Saunders, J.D. Mueller, G.W.G. Luxton, Fluorescence fluctuation spectroscopy reveals differential SUN protein oligomerization in living cells, Mol. Biol. Cell 29 (2018) 1003–1011, https://doi.org/10.1091/mbc.E17-04-0233.
[5] R.E. Goodchild, W.T. Dauer, Mislocalization to the nuclear envelope: an effect of the dystonia-causing torsinA mutation, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 847–852, https://doi.org/10.1073/pnas.0304375101.
[6] E.M. Smith, J. Hennen, Y. Chen, J.D. Mueller, In situ quantification of protein binding to the plasma membrane, Biophys. J. 108 (2015) 2648–2657, https://doi.org/10.1016/j.bpj.2015.04.021.