Frame sequences analysis technique of linear objects movement

V. Y. Oshchepkova, I. A. Berg, D. V. Shchepkin, and G. V. Kopylova

View Online   Export Citation

ARTICLES YOU MAY BE INTERESTED IN

Pulsating combustion: Theoretical and empirical substantiation of ecological effect
AIP Conference Proceedings **1910**, 020010 (2017); https://doi.org/10.1063/1.5013947

Regulation of the cooling power in the plunger cavity
AIP Conference Proceedings **1910**, 020004 (2017); https://doi.org/10.1063/1.5013941

Modeling of the optimal programs dependence on bodies geometric constraints when moving in a viscous medium
AIP Conference Proceedings **1910**, 020007 (2017); https://doi.org/10.1063/1.5013944

Frame sequences analysis technique of linear objects movement

V.Y. Oshchepkova[1, a)], I.A. Berg[1, b)], D.V. Shchepkin[2,c)], G.V. Kopylova[2, d)]

[1]*Ural Federal University, Mira 19, Yekaterinburg, Russia, 620002*
[2] *Institute of Immunology and Physiology, Pervomayskaya 106,Yekaterinburg, Russia 620049*

[a)] Corresponding author: valeoupi@gmail.com
[b)] berg77777@gmail.com
[c)] d.shchepkin@mail.ru
[d)] g_rodionova@mail.ru

Abstract. Obtaining data by noninvasive methods are often needed in many fields of science and engineering. This is achieved through video recording in various frame rate and light spectra. In doing so quantitative analysis of movement of the objects being studied becomes an important component of the research. This work discusses analysis of motion of linear objects on the two-dimensional plane. The complexity of this problem increases when the frame contains numerous objects whose images may overlap. This study uses a sequence containing 30 frames at the resolution of 62x62 pixels and frame rate of 2 Hz. It was required to determine the average velocity of objects motion. This velocity was found as an average velocity for 8-12 objects with the error of 15%. After processing dependencies of the average velocity vs. control parameters were found. The processing was performed in the software environment GMimPro with the subsequent approximation of the data obtained using the Hill equation.

INTRODUCTION

At present, there is a need for mathematical processing of experimental data in all fields of science including molecular physiology. Thanks to the development of modern visualization methods of the molecular interactions in vitro and in a living cell, as well as the improvement of computer technology, it became possible to carry out measurements using non-contact methods. To obtain experimental data, it is convenient to use video recording, because we can set the recording frequency, format and other parameters, that is significant for future data processing. When processing a video frame, we can easily scale the recording field. This allows simplifying the processing of experimental data and in some cases make it completely automatic. An actual example in which mathematical processing of the motion of linear objects is necessary is the study of the molecular mechanisms of muscle contraction in physiology.

At the molecular level, muscle contraction occurs due to the myosin interaction with actin of thin filament using energy of ATP hydrolysis. The actin-myosin interaction is regulated by calcium via regulatory proteins (troponin and tropomyosin) of thin filament. Mutations of contractile and regulatory proteins disrupt structure and function of its molecule and can lead to myopathy and cardiomyopathy. As an example, consider the study of the effect of tropomyosin mutations causing hypertrophy (HCM) and dilated (DCM) cardiomyopathies on calcium regulation of the actin-myosin interaction in skeletal muscle with an in vitro motility assay. At HCM, left ventricular wall and septal hypertrophy, myocyte disarray and interstitial fibrosis, increase the calcium sensitivity of the thin filament, systolic hypercontractility and impaired relaxation are observed. DCM is characterized increased left ventricular chamber volume, myocyte disarray, reducing the calcium sensitivity of the thin filament, decreased systolic function.

METHOD *IN VITRO* MOTILITY ASSAY

The *in vitro* motility assay allows studying actin-myosin interaction and its calcium regulation on the level of isolated contractile and regulatory proteins [1]. Using special equipment, we can visualize a movement of fluorescently labeled thin filaments consisting of F-actin, tropomyosin and troponin over myosin coated surface of a flow cell in present of ATP. This movement is a model of the actin-myosin interaction in muscle. Equipped includes epifluorescence microscope (Axiovert 200 M, Carl Zeiss MicroImaging GmbH) with the mercury lamp HBO 100 and a set of filters for fluorophore tetramethylrhodamine phalloidin (TRITC-phalloidin), high sensitivity iXon EMCCD camera (Andor Technology Ltd), and computer with special programs. The flow cell consists of two microscope glasses, one of them is cover glass with nitrocellulose surface.

In our study, myosin, actin and troponin are extracted from m. psoas of the rabbits [2-4]. Human α-tropomyosin (Tpm) with hypertrophic (E180G) and dilatated (E40K) cardiomyopathy mutations is expressed in E. coli [5]. Thin filaments are reconstructed from TRITC-phalloidin labeled F-actin, troponin, and Tpm. The experiments are done as earlier describes [5]. In brief, first to the flow cell, myosin is added and then bovine serum albumin is infused to cover the parts of nitrocellulose surface that are not covered by the myosin molecules. Further inactive myosin heads are blocked by F-actin with ATP and then fluorescently labeled thin filaments are added. To initialize the movement of thin filaments over myosin, buffer with ATP and appropriated calcium concentration is added. To obtain dependence of the filament sliding velocity on the calcium concentration we measure the velocity at different calcium concentrations from pCa 7.5 to pCa 4.0.

For this, the recording camera is configured. This study uses a series of 30 frames with the resolution of 62x62 nm/pixel recorded at the frame rate of 2 Hz, it is required to determine the average velocity of object motion. Images of flow cell areas 31.7x31.7 μm in size (the field) are recorded in a sequential order. Ten fields are recorded for each flow cell. Figure 1 demonstrates an example of obtained series for one field of the flow cell. Thin filaments are viewed as linear objects. Now we need to identify the linear objects, to find their velocities and dependence of its velocity from the calcium concentration.
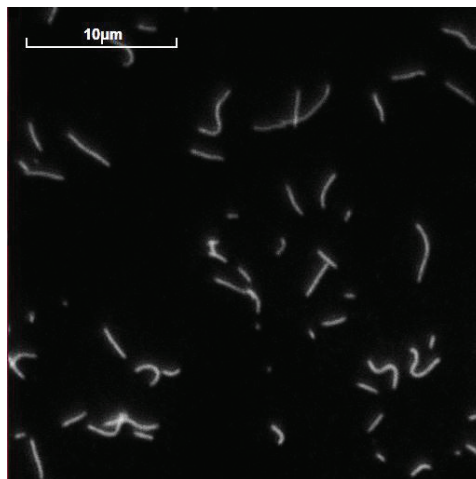

Figure 1. Fluorescently labeling thin filaments on the field of microscope.

DATA PROCESSING METHODOLOGY

Each object movement is complicated. It turned out to be impossible to process the series of raw data automatically because of limitation of the existing methods. For example, KochLab tracking application [6] uses image segmentation algorithms to identify microtubule ends via pattern matching. This software is unable to track microtubules with a segmented area of less than 55 pixels because of the matching algorithms used, and therefore the lower limit of the length of traceable microtubules is about 2 μm. Finally, tracking microtubule is stopped if the microtubule crossed other microtubules, or it too close to the edges of the field of view is located.

Previously our colleagues solved the problem of determining the velocity of fluorescent marked cytoplasmic and membranous associated protein molecules [7, 8]. For this it is necessary to identify one-dimensional objects

(illuminated spots within the cell). In the course of this study a math model was built. The model describes the motion of protein molecules in the cell. Based on that model a processing application GMimPro allowing automatically tracking one-dimensional objects and determining their velocities was developed [8].
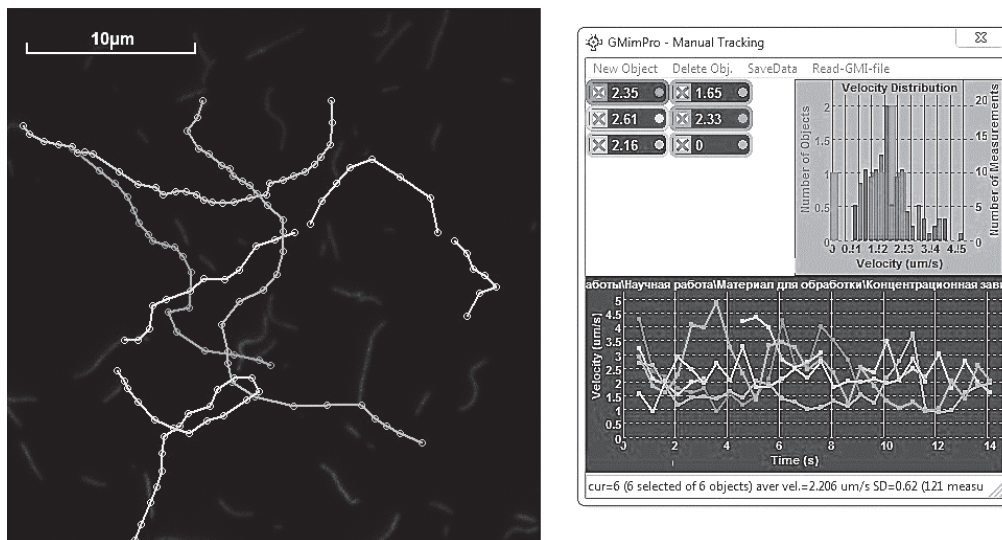

Figure 2. An example of window of GMimPro application with thin filaments in the field of the flow cell.

It was found that movement of a linear objects (thin filaments) it is possible to describe by the math model developed for studies of one-dimensional objects. To this end a methodology for processing obtained series for each field of the flow cell was developed. The GMimPro application interface during processing of linear objects movement is shown on the Fig. 2.

The processing were run following the procedure shown below:

1) The available sequences are fed into the software application GMimPro.
2) Manual tracking of each thin filament is performed. The last pixels in the direction of the object motion (that is the filament 'tail') is selected as a monitoring point.
3) Based on the standard deviation of the velocity determined by five – ten points (depending on the experiment), objects that do not meet the conditions are removed from the further processing. The standard deviation shall not be greater than 50% of the velocity of the given filament (a linear object), since the filaments that do not meet this requirement are functionally inadequate. If the filaments are moving at higher or lower velocity that exceeds or undermeasures the average velocity by 50%, that means that thin filament is damaged and the damage prevents it from normal functioning.
4) The average velocities of objects in the field is calculated by the GMimPro software application.
5) In a similar way, the average velocities of thin filaments in the remaining fields of the flow cell are found.
6) Based on the data found for one flow cell a 2D array is built. This array consists of the values of average velocities in each field and standard deviations for the fields.
7) Similarly, the data obtained when recording images of other flow cells with other calcium concentrations for a single muscle sample are processed. This leads to obtaining the dependence of the sliding velocity (or also force) on the calcium concentration $V = f(pCa)$ for each sample.
8) Dependence of the filament sliding velocity is sigmoidal. Approximation is made using the Hill equation: $V = V_{max}(1+10^{h(pCa-pCa50)})^{-1}$, where V and V_{max} are the velocity and maximal velocity (velocity at saturated calcium concentration), respectively, pCa_{50} (calcium sensitivity) is pCa at which the velocity is half maximal, h is the Hill coefficient.

Figure 3 shows result of processing: dependence of the sliding velocity of the regulated thin filaments over myosin on calcium concentration, the myosin, actin and troponin are extracted from muscle psoas of the rabbits.
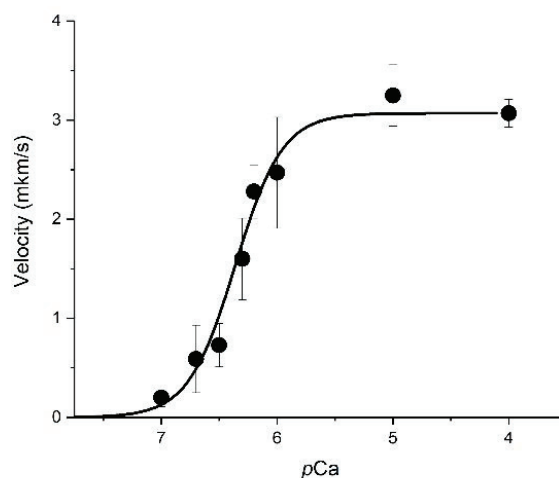
Figure 3. Example of the dependence of the sliding velocity of the regulated thin filaments over myosin on calcium concentration in the *in vitro* motility assay.

DISCUSSION AND CONCLUSIONS

Using data obtained with the in vitro motility assay and software GMimPro we analyzed the dependence of the sliding velocity of thin filaments containing Tpm with E40K or E180G mutations over skeletal myosin on the calcium concentration [9]. The parameters of the Hill equation, the maximal sliding velocity (Vmax) and calcium sensitivity (pCa50), characterize the impact of the changes in the structure of contractile and regulatory protein on contractile function of muscle. It was found that the DCM and HCM mutations in α-chain of Tpm have opposite effect on the calcium regulation of the interaction of skeletal myosin with thin filaments and its affect is similar to this previously obtained for the cardiac muscle. The HCM E180G Tpm mutation increases the maximal sliding velocity of thin filaments and calcium sensitivity. The DCM E40K mutation decreased both the sliding velocity and the calcium sensitivity.

This work can be important not only for development of fundamental knowledge on molecular mechanisms involved in functioning of the contractile apparatus of muscle both in normal and pathological conditions, but also it will help develop efficient pharmaceutical products that would prevent development of functional disorders in muscles induced by diabetes, thyrotoxicosis and etc.

ACKNOWLEDGMENTS

REFERENCES

[1]   Nikitina LV, Kopylova GV, Shchepkin DV, Nabiev SR, Bershitsky SY. (2015) Biochemistry (Mosc). 80(13):1748-63.
[2]   Margossian S.S. & Lowey S. (1982) Meth.Enzymol. 85: 55–71.
[3]   Pardee J.D & Spudich J.A. (1982) Meth Enzymol 85: 164-179.
[4]   Potter J.D. (1982) *Meth Enzymol* 85: 241-63.
[5]   Matyushenko AM, Shchepkin DV, Kopylova GV, Popruga KE, Artemova NV, Pivovarova AV, Bershitsky SY, Levitsky DI. (2017) Biochemistry. 56(1):250-259.
[6]   Maloney A, Herskowitz LJ, Koch SJ (2011) Effects of Surface Passivation on Gliding Motility Assays. PLoS ONE 6(6): e19522. https://doi.org/10.1371/journal.pone.0019522
[7]   Nenasheva TA, Mashanov GI, Peckham M, Molloy JE. (2011) Methods Mol Biol. 778:123-42.
[8]   Mashanov GI, Molloy JE. (2007) Biophys J. 92(6):2199-211.
[9]   Kopylova GV, Shchepkin DV, Borovkov DI, Matyushenko AM. (2016) Bull Exp Biol Med. 162(1):42-44.