HHS Public Access

Author manuscript

CSH Protoc. Author manuscript; available in PMC 2016 July 28.

Published in final edited form as:

CSH Protoc. ; 2007: pdb.prot4872.

Imaging Real-Time Gene Expression in Living Systems with Single-Transcript Resolution: Single mRNA Particle Tracking with ImageJ-Based Analysis

Amber L. Wells[1], **John S. Condeelis**[1,2], **Robert H. Singer**[1,2,3], and **Daniel Zenklusen**[1]

[1]Department of Anatomy and Structural Biology, Albert Einstein College of Medicine, Bronx, NY 10461, USA

[2]Gruss-Lipper Biophotonics Center, Albert Einstein College of Medicine, Bronx, NY 10461, USA

MATERIALS

Equipment

ImageJ software

Time-lapse movie frames or image stacks of fluorescent mRNA particles in living cells

METHOD

Maximum Image Projection

- 1. OPEN a file containing a stack of time-lapse frames from one movie or IMPORT individual frames as an “IMAGE SEQUENCE”. Figure 1 (top) shows the first frame of a movie stack used to create Movie 1.
- 2. Click on IMAGE from the menu bar, click on STACKS, and select Z-PROJECT.
- 3. Select MAX INTENSITY as the “Projection Type.” A new window opens that displays the brightest pixels from each time point. Upon inspection, particle paths should be evident, as seen in Figure 1 (middle).

Kymograph

- 4. Activate the window displaying the time-lapse movie stack.
- 5. Under the IMAGE menu item, click STACKS, and select RESLICE [/]. Keep the input and output spacing set to “1” and choose the location of the image from which it will start reslicing (top, bottom, left, or right). A new window will pop up, displaying the resliced planes in a stack.

[3]Corresponding author (rhsinger@aecom.yu.edu).

This protocol describes the use of ImageJ software (freely available from NIH) to analyze particle dynamics in a cell using time-lapse movie frames or image stacks of fluorescent mRNA particles. Maximum intensity projections and kymographs are produced.

- 6. Activate the Reslice stack window. Under the IMAGE menu item, click STACKS, and select Z-PROJECT. This displays a kymograph of the entire field by plotting position on the *x*-axis against time on the *y*-axis.

Figure 1 (bottom) shows an example of a kymograph derived from the maximum intensity projection image in Figure 1 (middle). Figure 1 (middle) was resliced starting from the top so that spatial information is retained on the x-axis. The y-axis shows the particle position from each frame of the time-lapse with t=0 at the top.

DISCUSSION

There are several excellent algorithms for object detection, centroid assignment, and localization as a function of time (Thompson et al. 2002; Gennerich and Schild 2005), which can identify and track many particles at once. These programs can distinguish diffusional motion and identify particle tracks that are nondiffusional based on mean square displacements of the centroids of diffraction-limited particles. Particle tracking plugins for ImageJ (MultiTracker and Particle Tracker) are freely available from the National Institutes of Health Web site (http://rsb.info.nih.gov/ij/plugins/). Software-based analysis systems track multiple paths and analyze their movements relatively quickly. However, due to the complexity of particle dynamics in live cells, they may mistake or miss paths that the human eye can see. Thus, visual verification of what the software has assigned as particles and tracks should be performed. Many parameters can be measured for moving particles, such as (but not limited to) diffusional or directed motions, velocities, and localization to cellular compartments or structures (Fusco et al. 2003). Identifying and recording the centroids of mRNA particles frame to frame from a time-lapse movie is time consuming. Trajectories are easily projected when the maximum fluorescence intensities (MFI) of all time points are displayed in one image. The MFI image can be further processed to obtain a kymograph, which shows the trajectories of the objects as a function of time. Particle speeds can be determined by calculating the slope of the lines in a kymograph. ImageJ performs the necessary steps to create a maximum intensity projection and kymograph. Figure 1 shows the analysis of MCP-GFP β-actin 3′UTR mRNA particle movements in a living cell over time (see Movie 1). Alternatively, an ImageJ plug-in can be downloaded that assembles the following steps into a macro. Briefly, one selects the “segmented line selection” to trace over a particle path displayed in a MAX INTENSITY image *z*-project window. The coordinates of the path specified using the line tool are recorded to calculate average and instantaneous velocities of the particle. The slope of the line corresponds to the average velocity of the particle. Directional changes are easily detected from kymograph displays, as are stationary particles (see the bottom of Fig. 1). Moving particles will have a slope other than 0. A stationary particle has a slope = 0 and will appear as a straight vertical line on a kymograph. Directed, corralled, diffusing, and stationary particles are easily visualized and quantified (Fusco et al. 2003).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

We thank Jason Dictenberg for critical reading of the manuscript and Shailesh Shenoy for helpful advice and discussions about the imaging system and particle analysis. This work is supported by the National Institutes of Health grants AR41480 (R.H.S.) and 5P01 CA100324 (J.S.C.).

References

Fusco D, Accornero N, Lavoie B, Shenoy SM, Blanchard JM, Singer RH, Bertrand E. Single mRNA molecules demonstrate probabilistic movement in living mammalian cells. Curr. Biol. 2003; 13:161–167. [PubMed: 12546792]

Gennerich A, Schild D. Sizing-up finite fluorescent particles with nanometer-scale precision by convolution and correlation image analysis. Eur. Biophys. J. 2005; 34:181–199. [PubMed: 15609049]

Thompson RE, Larson DR, Webb WW. Precise nanometer localization analysis for individual fluorescent probes. Biophys. J. 2002; 82:2775–2783. [PubMed: 11964263]

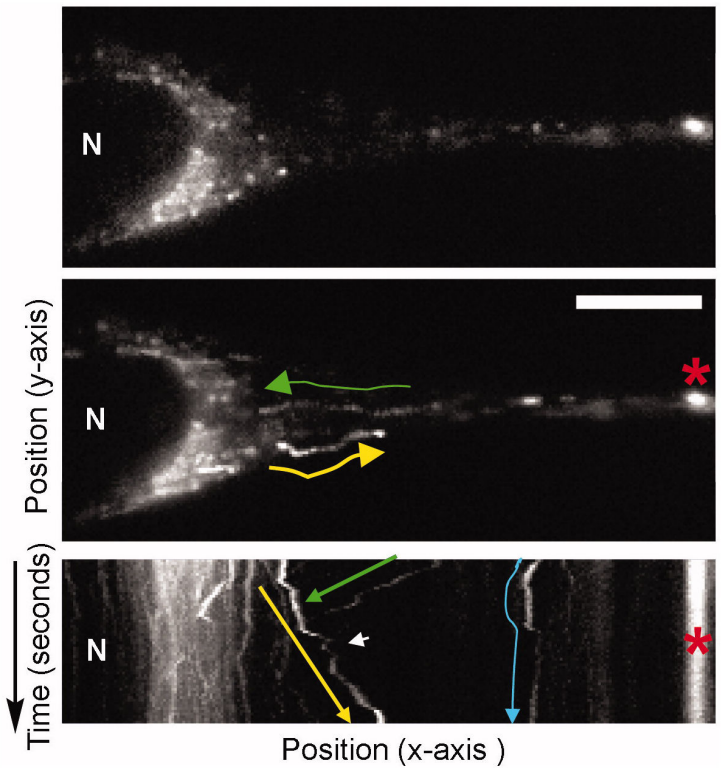

Figure 1.

mRNA particles exhibit multiple dynamic movements in a cell cotransfected with MCP-GFP-NLS and RSV-LacZ-β-actin 3′UTR (reporter RNA) plasmids. (*Top*) A still image of a movie sequence (see Movie 1) showing MCP-GFP bound to β-actin 3′UTR containing mRNAs in the cytoplasm of a cultured mammalian myoblast. (*Middle*) Maximum intensity projection of MCP-GFP particle trajectories (total time = 1 min.). Arrows indicate the direction of particle movement. (*Bottom*) A kymograph of the particle trajectories is shown with time increasing from top to bottom on the *y*-axis, and *x*-position changes with time on the *x*-axis. The slope characteristics show nondiffusional dynamics. (Red asterisk) Stationary particle with no velocity (*vertical line*). (Yellow arrow) Particle with nonuniform directed motility moving at an average of 0.15 μm/sec with a maximum instantaneous velocity of 1.52 μm/sec (at the white arrow). (Green arrow) Particle with directed motility averaging 0.45 μm/sec. (Blue arrow) Particle oscillating around a fixed point, identified as “corralled” movement. (*N*) Nucleus. Bar, 10 μm.