Systems biology

dNEMO: a tool for quantification of mRNA and punctate structures in time-lapse images of single cells

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Abstract

**Motivation:** Many biological processes are regulated by single molecules and molecular assemblies within cells that are visible by microscopy as punctate features, often diffraction limited. Here, we present detecting-NEMO (dNEMO), a computational tool optimized for accurate and rapid measurement of fluorescent puncta in fixed-cell and time-lapse images.

**Results:** The spot detection algorithm uses the à trous wavelet transform, a computationally inexpensive method that is robust to imaging noise. By combining automated with manual spot curation in the user interface, fluorescent puncta can be carefully selected and measured against their local background to extract high-quality single-cell data. Integrated into the workflow are segmentation and spot-inspection tools that enable almost real-time interaction with images without time consuming pre-processing steps. Although the software is agnostic to the type of puncta imaged, we demonstrate dNEMO using smFISH to measure transcript numbers in single cells in addition to the transient formation of IKK/NEMO puncta from time-lapse images of cells exposed to inflammatory stimuli. We conclude that dNEMO is an ideal user interface for rapid and accurate measurement of fluorescent molecular assemblies in biological imaging data.

**Availability and implementation:** The data and software are freely available online at https://github.com/recleelab.

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**Supplementary information:** Supplementary data are available at Bioinformatics online.

1 Introduction

Quantitative imaging of single cells enables measurement with subcellular resolution of dynamic biological processes that regulate critical cellular behaviors. Processes of the central dogma, such as active transcription, single mRNA transcripts, sites of active protein translation and other regulatory multi-protein assemblies can be observed by fluorescence microscopy as punctate structures within the cell (Femino et al., 1998; Larson et al., 2005; Lyon et al., 2019; Raj and van Oudenaarden, 2009; Tanenbaum et al., 2014; Wang et al., 2016). Spatiotemporal dynamics of signaling proteins, quantified in single cells by live-cell imaging, have revealed mechanistic insights into signal–response relationships in signal transduction networks in addition to sources of cell-to-cell variability (Gaudet and Miller-Jensen, 2016; Xu et al., 2005; Zhang et al., 2017a). However, most live-cell imaging approaches use fluorescent biosensors and fusion proteins that report within large subcellular compartments, such as the cytoplasm or nucleus, and quantification of punctate structures is often limited to fixed-cell and low-throughput applications. Accurate detection and quantification of biological puncta is necessary to examine their roles in regulating cellular behaviors, and computational analysis is often the rate-limiting step of experimental pipelines.

The nuclear factor (NF)-κB signal transduction pathway is a master regulator of inflammatory responses to injury and infection (Zhang et al., 2017b). Following activation of the pathway by inflammatory cytokines, such as interleukin-1 (IL-1) or tumor necrosis factor (TNF) among others, a series of intracellular signaling events transduce the NF-κB signal. Upstream kinase activation by the NF-κB essential modulator protein (NEMO, also known as IKKβ) is a necessary step in regulation of the classical NF-κB signaling cascade (Liu et al., 2012). Following cytokine stimulation, NEMO is recruited to polyubiquitin scaffolds associated with cytokine-ligated receptor complexes where NEMO-interacting IκB kinases (IKKs)...
are activated through induced proximity with other signaling media-
tors (Clark et al., 2013; Ea et al., 2006; Hayden and Ghosh, 2008; Pabon et al., 2019; Poyet et al., 2000). In cells that express EGFP fused to NEMO exposed to inflammatory cytokines, EGFP-NEMO transiently localizes to punctate fluorescent structures near the plasma membrane (Pabon et al., 2019; Tarantinò et al., 2014). The number and timescales of EGFP-NEMO-enriched puncta reveal quantitative properties of receptor-associated protein complexes that transmit information from the inflammatory milieu into the NF-kB transcriotional system.

Here, we present detecting-NEMO (dNEMO), a free application that uses wavelet-based spot detection and supervised segmentation to detect and measure properties of fluorescent puncta in fixed-cell and time-lapse images. In contrast with many applications that com-
pletely automate analysis, dNEMO encourages users to interact di-
rectly with their data and helps them to rapidly detect and measure fluorescence properties of puncta that are of interest. We show that the wavelet-based detection approach is significantly faster than traditional Gaussian fitting methods and allows for almost real-time interaction with single cells in quantitative imaging data. Intuitive tools for cell segmentation, spot inspection and background correction, in addition to manual and automated selection of puncta based on quantifiable features (e.g. size, location and fluorescence) ensure that single-cell data are of the highest quality. Results are formatted on quantifiable features (e.g. size, location and fluorescence) ensure that single-cell data are of the highest quality. Results are formatted for easy coordination with other software packages, such as single-
particle tracking applications and other analyses of structural dy-
namics (Jaquaman et al., 2011). Using smFISH and live-cell data for EGFP-NEMO as demonstrations, we show that dNEMO is a versa-
tile workspace for rapid, precise and robust measurement of fluores-
cence puncta in digital images.

2 Materials and methods

2.1 Live-cell imaging

At 24 h prior to imaging, U2OS cells expressing EGFP-NEMO from the endogenous gene locus described previously (Pabon et al., 2019) were seeded at 20 000 cells/well in complete growth medium on no.
1.5 glass bottom 96-well imaging plates (Matriplate). EGFP-NEMO expressing cells were imaged as z-stacks with an axial resolution of 0.5 μm using a 60× Plan APO objective (1.42 NA; Olympus) on a DeltaVision Elite microscope (GE Healthcare Life Science) equipped with a pco.edge sCMOS camera, Insight solid-state illumination module and environmentally controlled chamber (37°C, 5% CO2). Live cells were imaged for 30 min prior to stimulation and time-
lapping images were collected with a temporal resolution of 5 min per frame. At the time of stimulation, 120 μl of a pre-warmed mixture of recombinant human IL-1β (Peprotech) in growth was added so that the final concentration was 100 ng/ml.

2.2 The dNEMO user interface

The user interface is a MATLAB-based application which provides several means of interaction with single-channel images and movies. Users load a given image or movie into the application. The two over-arching processes of the application, cellular segmentation and puncta identification, are independent of one another and do not rely on each other to properly function. Puncta are identified by cre-
ating a keyframe to perform the wavelet transform over the frames of a time-lapse image. Further curation is handled using an inter-
active distribution of identified puncta within the current image and imposing limits based on spot size or intensity among other meas-
ured properties. Settings like the wavelet map level and the min-
imum number of consecutive slices of a 3D image, a spot must appear in are user-defined parameters. Once the user settings are estab-
lished for an experiment, the same settings are batch processed over all indicated images. A cell segmentation button in the interface initiates a process where the user defines a cell perimeter with an interactive polygon. The user then moves forward through the frames of a time-lapse movie and manipulates the polygon to define changing boundaries of the same cell. Once cells are segmented and puncta are localized, data for a single-cell time-course are automatically combined and stored and the number of puncta identi-
dified per cell is displayed in a graph in the lower right-hand corner of the dNEMO graphical user interface (GUI). A detailed view of identified puncta can be shown using a dedicated ‘spot inspector tool’, and spots erroneously identified by the wavelet transform (larger objects, vesicular artifacts) can be manually excluded with an interactive removal tool. Results can be saved and reloaded within the dNEMO interface for further analysis. All per-cell and per-spot trajectories are stored in the resulting output files in MATLAB and Excel-compatible formats.

2.3 The à trous wavelet transform

Implementation of the à trous wavelet algorithm is largely adapted from Izeddin et al. (2012). Briefly, the raw image is convolved with a matrix to create a wavelet map of the initial image. The L1 kernel is initially populated with values supplied by the third order B-spline (B3) (Unser and Aldroubi, 1992). As the level of the transform increases (L2, L3, etc.) zeros are inserted between each of the initial matrix values at increasing amounts. This effectively adds ‘holes’ in the convolution matrix with more zeros inserted as the level of the wavelet transform increases (see Supplementary Fig. S1). Subtracting the convolved image from the initial image produces a wavelet map. To identify puncta, a user-defined value is multiplied by the SD of the distribution of pixel intensities in the wavelet map to define a threshold. The threshold is used to classify pixels as ei-
ther background or foreground. Sub-threshold pixel values are set to 0 and considered background, foreground pixels are associated with puncta and analyzed further in the following watershed segmenta-
tion and analysis steps.

2.4 Watershed, thresholding and centroid detection

To determine centroids within each identified region within the wavelet map, we used the MATLAB (Mathworks) watershed func-
tion. Background is removed from the selected wavelet map (L1, L2, etc.) by setting pixel values below a user-defined threshold to zero. Thresholding is a manual procedure, and the dNEMO interface updates the source image in real-time to assist users with a first-pass visual estimation for puncta identified under the chosen threshold value (Fig. 2a). The remaining pixel values are multiplied by −1 to generate an inverted wavelet map so that foreground objects act as ‘catchment basins’ for the watershed transform. The spatial coordi-
nates for each basin and the surrounding path are used to define the centroid and perimeter, respectively, for each of the fluorescent punctum. For each punctate structure identified by the watershed transform, properties of interest are measured as defined by the user, including centroid location, size and intensity of the punctum, among others (see guide packaged with software).

2.5 Over-segmentation, 3D puncta localization and cura-

Fixed-cell smFISH microscopy images, previously created by Lee et al. (2014), simulated images and IL-1β-induced IKK puncta were used as experimental test cases for detection of fluorescent puncta. For analysis of puncta in 2D and 3D images, wavelet maps are pro-
duced for each 2D slice of the image stack. To resolve over-
segmentation, paths are generated for each pair of centroids that are separated by <10 pixels. Paths are scanned for a significant trough, defined by default as a pixel intensity value along the path that is at least 10% lower intensity than the endpoints of the path. If a significant trough is not found, the puncta are consolidated, and properties of the merged punctum are recalculated. Similarly, cent-
roids falling within three pixels of each other are combined because this is within the resolution limit of our optical imaging system.

For 3D images, centroids identified in each slice are referenced against centroids in adjacent slices and puncta are merged between slices of the image stack if the X-Y coordinates of their centroids fall within a Euclidean distance of two pixels. Fluorescence properties for each 3D punctum can be either aggregated across image slices or measured at the image slice that corresponds to the axial centroid.
User-defined bounds for the size and intensity of puncta can be used to filter puncta within a chosen range of fluorescence and size. In the user interface, filtered puncta are indicated with a red circle (Fig. 2c), which can also be hidden for visual clarity. The interface also provides tools for manual exclusion of erroneously identified puncta, and a spot-inspector tool to examine features of puncta in closer detail. Cell segmentation is independent from the detection and curation of puncta, and features are updated post hoc for each segmented cell if user-defined settings for spot detection are changed. Once user-defined settings are established, dNEMO is typically run in batch processing mode so that the methods for puncta detection are identical across all images in an experiment.

### 2.6 Local background correction

Background correction is performed locally for individual puncta in the source image. A binary mask is created representing the regions identified by the à trous wavelet transform and dilated (Hu et al., 2009) by a user-defined number of pixels (Supplementary Fig. S3a). Pixel intensity values are collected from the annular ring around each punctum and used as the punctum's local background. An additional user-defined parameter can be assigned to offset the inner diameter of the annular ring. The buffer region excludes the background pixels that immediately surround the punctum and may contain out-of-focus light from the fluorescent source. The background pixel values measured in the annular ring for a punctum are averaged and subtracted from each pixel identified within the punctum in the source image. To ensure accurate estimation of the local background, annuli pixels are not collected in the vicinity of other puncta (Supplementary Fig. S3b).

### 2.7 Cell segmentation

Segmentation of individual cells is performed by the user using an interactive polygon tool. This polygon can be further adjusted by the user in subsequent frames to account for morphology changes and cell movements over time. For compatibility with other segmentation tools, dNEMO can also import binary cell masks or the coordinates marking cellular perimeters for a given image. The procedure to import of cell segmentation data is described in the user manual packaged with the software. Cell segmentation uses the keyframing approach described above.

### 2.8 Simulated images with diffraction-limited objects

The 3D matrices of size $512 \times 512 \times 64$ ($X, Y, Z$) were populated with zeros. A polygon containing pixel values comparable with intensity values found in cellular regions in fluorescence microscopy images was inserted into every z-plane of each matrix to approximate slow-varying background fluorescence, such as unbound dyes or fluorescent proteins that do not reside in puncta. Pixel values within each polygon were increased moving from the polygon’s edges toward the polygon’s center and eventually plateaued at some constant value halfway between the polygon’s edges and centroid. A small Gaussian smoothing filter was applied to each pixel associated with the inserted polygon. Theoretical point spread functions (PSFs) were compared between images. We show both the uncorrected mean intensity and mean intensity corrected for local background (Supplementary Fig. S4e). The $R^2$ value for identified puncta is improved significantly (Supplementary Fig. S4f), demonstrating the accuracy for measurement of background-corrected puncta.

### 2.9 Comparison between dNEMO and FISH-quant

Simulated images containing theoretical PSFs approximating diffraction-limited objects of known coordinates and intensities were analyzed separately using dNEMO and FISH-quant (Mueller et al., 2013). Puncta were identified in both dNEMO and FISH-quant using the tools available in the ‘Spot Filter’ or ‘Spot Detection’ components, respectively. Additional post-processing of the puncta identified using FISH-quant was performed using the ‘Thresholding’ tool supplied in FISH-quant. Puncta were considered successfully identified if the measured centroid was within a Euclidean distance of two of the true centroids. Error rates for puncta localization accuracy were assessed against increasing amounts of simulated imaging noise. To generate noise, each pixel in the simulated image was corrupted with Gaussian noise of increasing standards of deviation (within the range of $[0, 300]$ SDs; Supplementary Fig. S4). For each simulated noisy image, the average signal-to-noise ratio was calculated by dividing the average signal intensity (defined as four pixels that constitute the centroid of the inserted PSF) by the SD of the background (defined as those pixels within the cellular region of the simulated image that are not part of the signal). Finally, the error rate was determined as the number of false positives and number of false negative puncta found in each image over the total number of signals present within the image (1500, 2500 or 5000 theoretical PSFs; Supplementary Fig. S4a and b).

### 2.10 Benchmarking dNEMO results

The same fixed-cell smFISH 3D image ($1024 \times 1024 \times 45$) image was analyzed with dNEMO and FISH-quant on a 2015 MacBook Pro laptop (16 GB RAM, 2.5 GHz processor) or an Intel® Xeon® PC (128 GB RAM, 2.3 GHz processor). Time durations were measured using native methods in MATLAB for the core punctum detection algorithms and fluorescence intensity quantification. Reported times do not include image pre-processing steps in FISH-quant (background correction and background region assignment, among others), image loading times or other user–GUI interactions. The smFISH image was analyzed five times in each application for statistical comparisons (Supplementary Fig. S4c).

### 2.11 Comparing smFISH in raw and deconvolved images

NFKBIA transcripts detected by smFISH in HeLa cells were obtained from a previous study (Lee et al., 2014) and were deconvolved with SoftWoRx using hardware specifications for the DeltaVision microscope (Applied Precision, GE Healthcare Life Science) used for the original image acquisition. Both images were analyzed with dNEMO and mean intensities for the same puncta were compared between images. We show both the uncorrected mean intensity and mean intensity corrected for local background (Supplementary Fig. S4e). The $R^2$ value for identified puncta is improved significantly (Supplementary Fig. S4f), demonstrating the accuracy for measurement of background-corrected puncta.

### 3 Results

#### 3.1 The à trous wavelet transform in dNEMO enhances fluorescent puncta

Wavelet-based approaches are used in image analysis for de-noising, compression and feature extraction with low computational cost (Akansu et al., 2010; Demirel and Anbarjafari, 2011). In wavelet-based feature extraction applications, the source image is decomposed into wavelet maps, a series of images where contrast is enhanced for particular spatial features. Since the wavelet transform sequentially applies a different convolution matrix at successive levels of the algorithm, the size and qualities of spatial features that are enhanced in each wavelet map can be modulated. To evaluate the effect of wavelet transformation, we used dNEMO to analyze three types of simulated and experimental images that contain diffraction-limited puncta (Fig. 1).
The à trous wavelet transform was previously used to detect isotropic diffraction-limited spots (Holtschneider et al., 1987; Izeddin et al., 2012). As the wavelet map transform level increases, zeros are progressively inserted into the à trous wavelet convolution matrix (Fig. 1a, see also Supplementary Fig. S1). Comparing experimental images of diffraction-limited spots at the first level of the wavelet algorithm (L1 wavelet map), noise and the smallest puncta in the source image were enhanced (Fig. 1b, second column). Consistent with previous findings (Izeddin et al., 2012; Olivo-Marin, 2002), the L2 wavelet map (Fig. 1b, third column; see also Supplementary Movie S1) enhanced contrast for puncta at or near the diffraction limit. At higher levels, larger puncta were more resolved (L3 wavelet map; Fig. 1b, fourth column) at the expense of reduced clarity for smaller puncta. Although users of dNEMO can select a wavelet map appropriate for their application, the L2 wavelet map effectively enhanced contrast in our three test images and was used to detect small molecular assemblies in subsequent experiments.

3.2 dNEMO detects and quantifies puncta in single cells

To identify fluorescent puncta near the diffraction limit, watershed and thresholding algorithms in dNEMO were used to segment wavelet maps for three types of imaging data. Puncta identified by watershed were then evaluated to prevent over-segmentation, where a single punctum in the source image (Fig. 2c; see also Supplementary Fig. S2). Instead of using procedural generation of annuli for each punctum, the method in dNEMO operates directly on the wavelet map and consequently background correction is rapid.

To associate and compare puncta between single cells, dNEMO contains an interactive polygon tool for manual cell segmentation (Fig. 2, middle left panel). Cell segmentation paths can also be imported into dNEMO for compatibility with results from other software. All puncta contained within the polygon are associated and puncta features, such as their number and distributions of fluorescent intensities among others, can be collated for each single cell (Fig. 2b). As a demonstration, we used dNEMO to detect single molecules of mRNA from smFISH images of TNF-induced expression of the NFKBIA gene using a previously collected dataset (Lee et al., 2014). Although there was significant variability in transcript numbers when compared between single cells, the size and fluorescence intensity distributions of puncta were similar (Fig. 3).

In the final analysis for 2D and 3D images, fluorescent and spatial properties are measured for each background-corrected punctum in each single cell. Spots detected by dNEMO agree strongly with those detected by eye and the approach resolves overlapping spots in 3D images that would otherwise be difficult to detect. Taken together, we find that dNEMO is effective at resolving near-diffraction-limited spots in imaging data.

3.3 Spot detection in dNEMO is rapid and accurate

In comparison with spot-fitting methods, such as 3D Gaussian or maximum likelihood estimation (Abraham et al., 2009), the wavelet-based approach in dNEMO does not require iterative estimation of parameters or image pre-processing steps. To compare against our application, we selected the software package FISH-quant (Mueller et al., 2013) primarily because it implements a 3D Gaussian fitting method for detection of transcripts and it is actively used in the research community. For example, we had previously used FISH-quant to detect single mRNA molecules in the context of stochastic transcription events (Wong et al., 2018).

Although the localization accuracy and runtime of the à trous and Gaussian fitting methods have been compared at the level of the algorithm (Izeddin et al., 2012), we set out to make a practical comparison.
comparison of applications using simulated and fixed-cell data. To
test both applications, we generated noisy experimental images
using a theoretical PSF to simulate diffraction-limited fluorescent
puncta in a slow-varying background with varying amounts
of Gaussian noise (top row, Fig. 1b; Supplementary Fig. S4a; see also
Section 2). We compared localization accuracy for dNEMO and
FISH-quant using the same sets of simulated images. With ground
truth information for the position and number of puncta in simu-
lated data, we found that both applications have almost negligible
localization error in low noise, but error rates for dNEMO remained
lower for images with greater noise (Supplementary Fig. S4b). Wall-
clock time for single core performance using whole-image smFISH
data showed that dNEMO is over 15-fold faster than FISH-quant
on a modern laptop computer (Supplementary Fig. S4c), with an
estimated 80% elapsed execution time dedicated to the over-
segmentation algorithm.

We finally compared accuracy of the background-corrected in-
tensity for detected puncta by comparing intensity values for a raw
image with a deconvolved image. Deconvolution is a computa-
tionaly expensive pre-processing approach that redistributes out-of-focus
light in a 3D image, thereby increasing the effective image resolution
and precision of intensity measurements. \textit{$R^2$} values demonstrated
that background corrected intensity values for raw and deconvolved
images (Supplementary Fig. S4d) are almost equivalent, and signifi-
cantly greater than uncorrected images (\textit{$R^2$} values of 90 and 58, re-
spectively; Supplementary Fig. S4c). We therefore conclude that
image pre-processing steps, such as deconvolution do not necessarily
increase the relative accuracy of dNEMO intensity measurements in
arbitrary units. Taken together, when images are collected in the lin-
ear regime of an imaging sensor, such as the sCMOS used here, spot
quantification in dNEMO is rapid, accurate and robust to noise in
imaging data.

3.4 Keyframing to detect time-varying features of
puncta in time-lapse images

Keyframing is a process in animation that denotes the start and end
frames in a time series where parameter values change. To enable
analysis of time-lapse images, dNEMO uses a keyframing approach
for users to make changes to any user-defined parameter and to
track single cells in time-lapse experiments. For example, a user may
define a region of the time-lapse where the wavelet map threshold or
bounds for acceptable puncta are modified to account for systematic
artifacts, and keyframing parameters can be applied in batch across
all images in an experiment. A more common use for keyframing in
dNEMO is to adjust the segmentation polygon to account for
morphology changes and tracking of a cell’s movement over the
time-lapse image (Fig. 2, bottom panel).

To demonstrate keyframing, we analyzed a 3D time-lapse image
of CRISPR/Cas9-modified U2OS cells that express EGFP-tagged
NEMO from its endogenous gene locus in response to IL-1 (Pabon
et al., 2019). Formation of NEMO puncta in single cells were
tracked by making keyframe adjustments to cell segmentation poly-
gons (Fig. 4a; see also Supplementary Movie S2). Fluorescent prop-
erties of NEMO puncta were followed over the time-lapse to
monitor time-courses for adaptive changes in NEMO puncta. Our
results demonstrate that the numbers of NEMO puncta in addition
to measurement for spot-to-spot variability of NEMO puncta over time in each cell (Fig. 4b). Taken together, distributions for properties of fluorescent puncta can be accurately measured and curated in time-lapse images to produce high-quality datasets in each single cell.

4 Discussion

In this work, we have shown that dNEMO is an effective tool for quantification of fluorescent puncta in fixed-cell and time-lapse images. The *a trous* wavelet algorithm progressively removes high frequency noise within fluorescent images and can be used to enhance puncta near the diffraction limit and larger. When compared with established methods, dNEMO performs with comparable or better localization accuracy, depending on the amount of noise in the source image, and is significantly faster. Keyframing in dNEMO provides an effective interface to curate single-cell data and correct for systematic effects in imaging data. We demonstrate dNEMO using fixed-cell smFISH and live-cell enrichment of EGFP-NEMO to puncta and expect dNEMO will also excel at quantifying other fluorescence reporters, including components of the central dogma, protein assemblies and bright vesicular structures.

The detection method’s speed and robustness to typical imaging noise made the *a trous* wavelet algorithm ideal for an interactive application like dNEMO, but we acknowledge limitations exist with the approach. Chief among the limitations is the *ad-hoc* threshold for the wavelet map, which must be defined by the user and is therefore subjective. Although dNEMO is suitable for detection of relatively bright structures in epifluorescence images, model fitting may still be the preferred method for detection of structures with lower signal-to-noise (Aguet et al., 2013; Loerke et al., 2009). Gaussian models use statistical tests to remove manual intervention and can discriminate between criteria for shape and size, which may be preferred for single-particle detection and similar applications.

Updates to dNEMO are expected to further reduce its runtime and enhance its capabilities. One notable limitation of the current dNEMO implementation is the disproportionate amount of overhead dedicated to the over-segmentation algorithm. We expect that these can be mitigated through updates for parallelization of the over-segmentation process or by modifications to the watershed algorithm that reduce the computational expense while maintaining accuracy. Beyond runtime improvements, one of the largest bottlenecks is the manual segmentation of cells. We are actively considering experimental methods for labeling and incorporating an automated cell segmentation approach into dNEMO, either directly or through a plug-in system where users can choose their own cell segmentation method. Finally, we are also streamlining dNEMO data structures for compatibility with existing single-particle tracking packages (Jaqaman et al., 2008), so that time-varying properties of single puncta can be tracked and associated with single-cell responses.

Tools dedicated to the processing of biological images have enabled many studies of single-cell variability and dynamics, and contributed to the discovery of emergent cellular properties. dNEMO fills a gap in the scientific community by providing a simple workspace for users to interact with biological puncta in fluorescence microscopy images that are central to fundamental cellular processes. The software is controlled with a MATLAB user interface or as a stand-alone executable, and is available as Supplementary Software or at https://github.com/recleelab along with a user manual and test data used to generate the figures in this article.

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Conflict of Interest: none declared.

Data availability

The data underlying this article are available in the article, its online supplementary material, and at https://github.com/recleelab. Data will also be shared on reasonable request to the corresponding author.

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