Published in final edited form as:

Methods. 2019 March 15; 157: 15–27. doi:10.1016/j.ymeth.2018.10.009.

A versatile image analysis platform for three-dimensional nuclear reconstruction

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Abstract

Nuclear morphology is indicative of cellular health in many contexts. In order to robustly and quantitatively measure nuclear size and shape, numerous experimental methods leveraging fluorescence microscopy have been developed. While these methods are useful for quantifying two-dimensional morphology, they often fail to accurately represent the three-dimensional structure of the nucleus, thus omitting important spatial and volumetric information. To address the need for a more accurate image analysis modality, we have developed a software platform that faithfully reconstructs membrane surfaces in three dimensions with sub-pixel resolution. Here, we showcase its broad applicability across species and nuclear scale, as well as provide information on how to employ this platform for diverse experimental systems.

Keywords

Nucleus; nuclear envelope; nuclear morphology; image analysis; 3D reconstruction

1. Introduction

The nucleus is a complex cellular compartment, and its size, shape, and mechanical properties are tightly regulated to ensure its proper functions [1–4]. The physical segregation of the nuclear compartment imparted by the nuclear envelope helps ensure protection of the genome, maintains the nuclear proteome by controlling the passive and facilitated transport of vital proteins involved in transcription and genome integrity, and provides a mechanism by which the innate immune system can recognize foreign but not self-DNA [5,6]. Aberrant nuclear morphology can therefore be an indicator of cellular dysfunction and has been widely used as a hallmark of pathology [7–11]. Cancer cells commonly exhibit altered

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nuclear morphology and scale, while morphological changes are also observed in other systemic human disease such as progeria and muscular dystrophy [12–14].

Many approaches have been developed for quantitatively assessing nuclear size and shape, including measurements of nuclear cross-sectional area [15–17], circularity [18], contour aspect ratios [14,19,20], irregularity [21], and mean negative curvature [12,13]. These methods yield important two-dimensional (2D) insight but are not able to fully characterize the complex, three-dimensional (3D) morphology and volume of the nucleus. In general, imaging nuclei in three-dimensions presents unique challenges. Rarely are nuclei perfect spheres, especially in mammalian systems, where a significant degree of asymmetry exists between the contours of the apical and basal aspects of the nucleus. For example, cultured mammalian cells adhered to a stiff surface like glass or plastic typically display nuclei that are nearly flat on the basal surface, while the apical surface varies from flat to more rounded, depending on the cell type and plating conditions. In either case, assuming spherical proportions will substantially overestimate the nuclear volume from a 2D cross section. Furthermore, measurements that account for deviations from circularity usually only examine the cross-sectional contour and do not account for volumetric changes or deviations at the distal surfaces or “caps” (top and bottom in *z*) of the nucleus. In particular, spherical regions where the contour shape is changing rapidly in *z* (likely at the apical surface) are challenging to faithfully capture; indeed, most investigators typically obtain spatial information on localization at the nuclear envelope on the basal (flat) nuclear surface or in the plane bisecting the nucleus. However, accurate nuclear reconstruction must recover the complete *z*-dependence of the nuclear contour to be reliable.

In an effort to provide a robust image analysis platform that addresses these challenges and more accurately assesses 3D nuclear morphology and volume, we previously developed software that uses fluorescent images to reconstruct the nucleus in three dimensions with sub-pixel resolution, employed initially in yeast model systems [22]. Using this approach in fission yeast, we are able to robustly calculate nuclear volume as well as to determine morphological changes of the nuclear envelope over time [23]. Here, we have further developed this software for use across a wide range of nuclear scale and shape—from small, spherical yeast nuclei to large, non-spherical mammalian nuclei—both in fixed and live-cell systems. We discuss a general approach to imaging and analyzing nuclear shape and nuclear envelope fluctuations using our platform, most concepts of which are transferable to many other image analysis modalities.

2. Image analysis methodology

2.1 Sub-pixel reconstruction resolution using maximum a posteriori (MAP) estimation

The standard method of constructing a 3D representation from 2D images fails to accurately represent the membrane surface between each plane of a *z*-stack and is especially deficient when assessing the membrane surface at the distal poles along the *z*-dimension in spheres (e.g., the top and bottom “caps”). As a result, the overall morphology is inaccurately depicted, and the total interior volume inaccurately calculated. To overcome this loss of spatial information and allow for more accurate measurements, our software uses maximum a posteriori (MAP) estimation to fit a *z*-stack of fluorescent images to a 3D mesh, which

represents the membrane surface. Specifically, the reconstruction algorithm seeks to optimize the description of the experimental fluorescence intensity in a z-stack without introducing unreasonably large curvatures into the reconstructed surface. A key aspect of the algorithm is a regularization parameter, which acts like a surface bending modulus, whose value is defined by the user.

Maximum a posteriori (MAP) estimation uses a Bayesian probabilistic approach to determine the appropriate mesh from a given z-stack of 2D images. The algorithm aims to find the highest probability of the mesh being a certain shape. This goal is achieved by relating this (posterior) probability to the product of 1) the probability of realizing the current mesh with 2) the likelihood of the same z-stack of images producing this mesh. If the resulting probability is low, the algorithm will iteratively improve the mesh location values to maximize the probability that the final 3D mesh is a faithful representation of the membrane contour, given the measured image stack.

In practice, organelles that are predominantly spherical are initially fit to a predefined spherical 3D triangle mesh. To tune the contour mesh to accurately represent the actual membrane contour details, organelle shapes that deviate from spherical are dynamically fit with a 3D triangle mesh generated from an initial, lower-resolution mask derived from the individual voxel locations of the fluorescently labeled membrane. In both cases, the surface contour is then optimized using MAP to achieve sub-pixel contour location precision. (For a more detailed description of this optimization approach, see [22]). The resultant surface reconstructions can then be used to qualitatively and quantitatively assess the 3D morphology, including measuring surface area and volume, and tracking changes in morphology and size when used in conjunction with time-lapse movies of live cells, which can provide greater insight into the mechanical properties of the nucleus [23].

2.2 Reconstruction parameter optimization and error quantification

To quantify the accuracy of the reconstruction for a specific fluorescent tag and biological sample, we recommend a simulation approach. For example, one can simulate a series of fluorescent images where a nucleus with a known size has been labeled with a specific fluorophore. After verifying that this simulated nucleus has a similar fluorescence intensity profile to an experimental nucleus, one can adjust the bending modulus of the reconstruction algorithm to track its effect on the method's ability to faithfully reconstruct the known ("ground truth") surface. The optimal bending modulus—or regularization parameter—should be large enough to suppress imaging noise contributing to the contouring, yet small enough to maintain sensitivity to finer deformations; thus, the ideal regularization parameter will give the smallest error between the reconstructed nucleus and the ground truth contour. Similar simulations can be performed for other fluorescent markers of interest to quantify their adequacy for use with the reconstruction software. Thus, this quantification allows one to select the optimal fluorescent marker and regularization parameter for the reconstruction as well as assess the minimum error achievable with these conditions. A more detailed description of how image noise affects the bending energy and overall reconstruction accuracy is discussed in Section 3.1, and further descriptions of this simulation and optimization procedure can be found in [22], Supplemental Information.

3. Sample preparation

3.1 Choice of fluorescent marker

To maximize the precision of the reconstruction software, choosing an appropriate fluorescent nuclear envelope marker is essential. The software can successfully reconstruct nuclear shape using a range of fluorescent nuclear envelope or endoplasmic reticulum markers, but optimal reconstruction precision is achieved when the fluorescence signal contrast, or signal-to-noise ratio (SNR) is high. This optimization can be achieved by selecting a fluorescent protein or antibody that is both selective and abundant at the nuclear envelope. For example, Nup82, a nuclear pore complex (NPC) protein (or nucleoporin) can be tagged with green fluorescent protein (GFP) to label the nuclear envelope in budding yeast and exhibits an excellent signal-to-noise ratio (Fig. 1A–C). In contrast, a GFP-fusion of the integral inner nuclear membrane protein Heh1 (a member of the LEM domain family) can be used to label the nuclear envelope, but because its abundance at the nuclear envelope is relatively low—as compared to a nucleoporin—the resulting fluorescent signal is correspondingly more difficult to differentiate from the background fluorescent signal (Fig. 1D–F) than in the case of Nup82-GFP.

As the image contrast decreases, the reconstruction software has increased difficulty in finding the nuclear envelope in the two-dimensional z-slices (see discussion on canny edge detection in Section 3.2). Low image contrast also affects the software's ability to detect small details in the membrane surface, therefore decreasing its accuracy. Figure 2 highlights this effect in comparing the nuclear surface contour intensity normalized to the mean image fluorescence (Fig. 2A) and the bending energy used to account for small deviations in shape (Fig. 2B). Lower total contour signal intensity results in higher bending energies used and indicates an over-suppression of image detail. In both plots, we compare Heh1-GFP (green) and Nup82-GFP (blue) from Figure 1 as well as Cut11-GFP (black; the prototype upon which the software was built). By comparing to the prototype fluorescent marker, one can assess the relative error in contour determination by referring to Supp. Fig. 1D in [22]. The minimal error achievable by the reconstruction software is proportional to the regularization parameter (i.e., the bending modulus). For surface contour intensities and membrane bending energies similar to a Cut11-GFP nucleus, the error is between 25 and 30 nm. Contours with lower normalized intensity—and thus higher bending energies (Fig. 2: Heh1-GFP; green)—will have decreased sensitivity to small contour deviations and larger error in their reconstructions.

Figure 3 shows the nuclear envelope reconstructions for Nup82-GFP and Heh1-GFP expressing nuclei from Figure 1. Traces of the reconstructed contours can be visualized on single 2D z-slices (Fig. 3A, E). The reconstruction software is able to resolve the Nup82-GFP-labeled membrane surface throughout the z-stack series (Fig. 3A) and is able to reconstruct the nuclear envelope (Fig. 3D). In contrast, the reconstruction software has difficulty tracking and reconstructing the Heh1-GFP nuclear envelope contour (Fig. 3E–H) due to the lower image contrast (Figs. 1–2), ultimately underestimating the nuclear volume and poorly resolving details of the nuclear surface due to the high bending energies (Fig. 2B). It is worthy to note that for a given image z-stack, the software is able to resolve a

portion of Heh1-GFP nuclei, but many nuclei are excluded due to the complications from low image contrast. With an optimal fluorescent marker such as Nup82-GFP or Cut11-GFP, however, the software is typically able to reconstruct all of the nuclei contained in the z-stack series. Thus, using an optimal marker will not only ensure reconstruction accuracy but will also increase the efficiency of the software.

In general, we have found the following fluorescent reporters work well with our reconstruction software (see Table 1), but other markers could be optimized for use, provided their abundance at the organelle membrane is high enough to ensure a robust signal-to-noise fluorescence ratio.

Another important consideration is the specificity of the fluorescent reporter to tag the membrane of interest. For example, if the fluorescent marker of choice labels the endoplasmic reticulum (ER) in addition to the nuclear envelope, this will affect the precision of the nuclear contour. A reconstruction is still possible, but the accuracy of the contour position—and thus the volumetric measurements—will be somewhat compromised. Figure 4 demonstrates the software's ability to reconstruct an *S. cerevisiae* nucleus labeled with the ER-resident protein, Hmg1-mCherry. Nonetheless, for certain experiments, for example when expression of a fluorescently-tagged nucleoporin might introduce unwanted genetic interactions with other proteins of interest, use of an alternative, non-nucleoporin marker to reconstruct the nuclear membrane is still possible.

3.2 Important microscopy considerations

To ensure accurate image reconstruction, there are a few vital data acquisition parameters to optimize when imaging samples. First, samples may be imaged with either a wide-field or confocal microscope, and acquired images should not be deconvolved or processed before inputting into the reconstruction software. Second, z-stack images should be acquired at the highest possible resolution of the imaging system, both in x, y, and z dimensions. For conventional, diffraction-limited fluorescence microscopy, z-slices of 0.2 μm are ideal as this affords the finest spatial resolution along z, which is the most difficult dimension to resolve and reconstruct because of the lack of spatial information between z-slices and because of out-of-focus fluorescence emanating from other z-planes, which contributes noise. However, in some experiments where fast image acquisition is required (e.g., for time-lapse movies), z-slices of 0.4 μm can be used with the software, leading to z-stack acquisition that is twice as fast (Fig. 5). The trade-off is that, although these z-stacks can still produce high-resolution reconstructions, they are not the highest-resolution possible.

Third, spatial drift in all three dimensions must be minimal. This is important for all imaging contexts but especially for time-lapse movies that will be used to reconstruct a sequence of contours from individual nuclei.

Fourth, in order to sufficiently reconstruct the entire height of the organelle, the z-stack must span beyond the upper and lower boundaries of the fluorescent membrane signal by at least two z-slices. The reconstruction software is automated to fit and reconstruct any nucleus in the image field of view that falls within predefined or user-specified parameters (e.g., predicted size of object in pixels), however, if it cannot completely fit the total height of the

nucleus within the z-stack (see Figs. 3B, 4B, 5B), the software will not attempt a reconstruction of that nucleus.

Fifth, images should be written using the most information-rich bit system (i.e., 16-bit rather than 8-bit images). Higher bit-size images provide greater pixel intensity resolution, which translates to more accurate initial surface meshes for MAP estimation after background subtraction and more accurate canny edge detection of the membrane boundary (Fig. 6A–C and Supp. Fig. 1). In lower bit-sized images, 2D membrane information is lost after background filtration and canny-edge detection (Fig. 6DA–F). If only lower bit-size images are available, it has proven possible to partially compensate by adjusting to increase the image contrast before passing the images on to the reconstruction software (Appendix 1 and Fig. 6GA–I).

Finally, organelles to be imaged and used for reconstruction should be spatially resolvable from other fluorescently-labeled domains. If two or more nuclei are spatially indistinguishable by eye (i.e., their boundaries overlap in x, y, or z), the reconstruction software will also have difficulty in separating them and will inaccurately reconstruct their boundaries. If possible, samples should be imaged avoiding crowding and overlap. These conditions can be achieved by ensuring that samples are adequately dilute, and that the slide preparation minimizes crowding in z (e.g., cells stacked on top of each other). If a certain amount of crowding is unavoidable due to the nature of the biological system—such as in cell colonies, organoids, or tissue specimens—a simple filtering algorithm may be employed after image acquisition and before image reconstruction (Appendix 2 and Fig. 7). This program allows one to hand-select which membrane(s) to include and exclude in the reconstruction. Elimination of unwanted, nearby fluorescent intensity allows for more accurate 3D mesh fitting and reconstruction (Fig. 7E).

Implementation of these imaging tips and tools allows the reconstruction software to be applied over a broad range of experimental systems. As a further example, Figure 8 showcases 3D reconstructions of mouse induced pluripotent stem (iPS) cell nuclei, which exist in close proximity to one another, and, depending on the conditions, may have shapes that deviate significantly from spherical. This type of application of the membrane reconstruction software on more complex mammalian samples highlights the platform's versatility and usefulness in many experimental systems and contexts.

4. Image analysis

4.1 3DMembraneReconstruction software setup

The most up-to-date version of the reconstruction software is available for free download from Zenodo (DOI: [10.5281/zenodo.1316957](https://doi.org/10.5281/zenodo.1316957)). Subsequent software updates can be found on GitHub (<https://github.com/mochrielab/3DMembraneReconstruction>). We recommend forking the master repository and cloning it to your desktop; this will give the user the freedom to make changes to the code while maintaining the ability to revert these changes back to the original format and also sync your forked repository to the master if software updates are available.

The 3DMembraneReconstruction software was written to run optimally on MATLAB versions 2015a-2016b. Ensure that one of these versions is installed and operating. Before running the software, add the 3DMembraneReconstruction folder to the MATLAB path. If using the user interface (UI), navigate to the main.m program and run it to initialize the analysis. If using the manual code (sample_script_membrane.m), navigate to the image file locations. Further detailed instructions on running the software are provided in the source code on GitHub.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We wish to acknowledge Drs. Peter Koo and Yao Zhao, who developed the MAP approach; Drs. Paolo Colombi, Rachel Stewart, and Shangqin Guo, who obtained raw images used in the example reconstructions shown here; and Dr. Eduardo Torres who provided yeast strains. This work was supported by the National Science Foundation (CMMI-1334406), the Raymond and Beverly Sackler Institute for Biological, Physical and Engineering Sciences, and the National Institutes of Health (T32-GM007223).

Appendix

1. Contrast adjustment compensation for lower bit-sized images

To compensate for lower bit size, images may be adjusted to increase the range of pixel intensities represented (i.e., increase contrast). A simple MATLAB procedure to achieve this uses the function `imadjust`, which redistributes pixel intensity so that the extreme low and high intensity values are only 1% of the represented pixels. A sample script for image contrast adjustment is as follows:

```
for i = 1:size(wimg3,3)
    wimg3_adj(:, :, i) = imadjust(wimg3(:, :, i));
end
```

% adjust pixel
% intensity to increase
% overall image contrast.

2. Manual boundary exclusion to prevent incorrect membrane reconstruction in dense nuclear samples

In order to use the reconstruction software on denser nuclear samples (e.g., stem cell colonies, organoids, and whole tissue), one may need to manually exclude unwanted fluorescent boundaries. The following MATLAB script (`excludeRegions.m`) is a simple method for boundary exclusion that removes unwanted regions of interest throughout the z stack of processed images.


```

function [img_filt] = excludeRegions(obj,regionNum)
    for k = 1:regionNum % Loop for multiple regions in image to be
                        % excluded.
        midplane = round(length(obj)/2); % Find midplane of z-stack.

        mask = roipoly(obj(:,:,midplane)); % Use polygon tool to
                                           % draw polygon around
                                           % region to be excluded.
                                           % When finished closing
                                           % polygon, double click
                                           % in center of polygon
                                           % to save polygon mask.

        if k == 1
            img_filt = obj; % Create image copy to be filtered.
        end

        figure
        imshowpair(img_filt(:,:,midplane),mask) % Show mask on top of
                                                % original image to show
                                                % the region to be
                                                % converted to zeros.

        [mask_y, mask_x] = find(mask); % Save mask [row, column]
                                       % coordinates as separate vectors.

        for j = 1:length(img_filt) % For each z slice...
            for i = 1:length(mask_x) % For each mask coordinate...
                img_filt(mask_y(i),mask_x(i),j) = 0; % Convert the
                                                    % corresponding image
                                                    % coordinate value to
                                                    % zero.
            end
        end

        figure
        imshow(img_filt(:,:,midplane)) % Show filtered image result.
    end
end

```

3. Batch processing for multiple image sets

A convenient addition to the software was recently added to allow for batch processing of multiple image sets imaged using the same parameters. Batch processing allows for a series of images to be processed and analyzed in parallel. The following is a detailed description of how to run the batch processing code.

After adding the updated 3DMembraneReconstruction to your MATLAB path, open the script “batch_processing.m”, which can be found in the 3DMembraneReconstruction parent folder. Make sure all of your movie/data files are stored as follows:

1. Each movie/file set is in its own folder.
2. Each of these folders are in one parent folder.
3. This parent folder is open in MATLAB’s Current Folder (displaying all the movie set folders) (for setup help, see accompanying setup documentation)

included in the 3DMembraneReconstruction download on GitHub: <https://github.com/mochrielab/3DMembraneReconstruction>).

The first section of the code includes all of the processing parameters one would like to apply to all of one's movies/datasets. Read through each variable and make sure it is consistent with the imaging conditions (e.g., number of imaging channels, name of the fluorescent particle, etc.).

```
%% 3DMembraneReconstruction Batch Processing
% Section #1

% This code processes a series of movies in parallel to allow for quicker
% analysis. The same steps are executed here as in the GUI and
% sample_script.m.

% Note: all movies to be processed should have the same parameters. This
% batch code will simply iterate the same parameters over each movie.
```

Follow these steps:

```
% To run:
% 1) Ensure all movie sets to be processed are in their own folder,
% and these folders are in the same parent folder, and this parent
% folder is open in the Current Folder.
% 2) Fill out and check all of the processing parameters below.
% 3) Run this section (Section #1) to load variables into Workspace.
% 4) Run Section #2 to process all the datasets together.

% Jessica Johnston // last edited February 8, 2018.
```

READ AND FILL OUT

```
% SET UP PROCESSING PARAMETERS - ** edit & check **

% Clear workspace, et cetera.
clear all;close all;clc;

% What type of images are to be processed? Example, '*.tif', or '*.dv'
image_type = '*.tif';

% **** CHANNELS ****

% How many channels are in image stack?
num_channels = 4;

% Which channels are to be used for the reconstruction analysis? If yes,
% assign to '1' for particle channel and '2' for membrane channel. If not
% using channel, assign to '0'.
channel_1 = 0;
channel_2 = 0;
channel_3 = 1;
channel_4 = 2;

% **** PARTICLE ANALYSIS ****

% For one particle analysis, set to '1'; for two particle analysis, set to
% '2'; to omit this analysis altogether, set to '0'.
particle_channel = 1;
% Designate particle name(s).
particle_name = 'loci';
% particle_name2 = 'mCherry-loci';
% Designate particle size in pixels (approximate).
particle_size = 3;

% **** MEMBRANE ANALYSIS ****

% For membrane analysis, set to '1'; to omit this, set to '0'.
membrane_channel = 1;
% Designate membrane name.
membrane_name = 'cut11';
% Designate membrane size in pixels (approximate).
membrane_size = 30;

% **** CELL CONSTRUCTION ***

% Designate how to construct the cells - by particles only, membranes only,
% or by both particles and membranes. If yes, set to '1'; if no, set to
% '0'. Only set one variable to '1'; leave other two variable as '0'.
% Construct cells only by particles:
cell_construct_particle = 0;
% Construct cells only by membranes:
cell_construct_membrane = 0;
% Construct cells by both particles and membranes:
cell_construct_membrane_particle = 1;

% How many fluorescent particles should be in each cell? Set this number as
% a means of filtering out cells with fewer/more particles.
particle_number = 1;

% **** SAVING IMAGES ****

% Show and save each reconstruction? If so, set 'show_and_save' to '1'; if
% you want to run the reconstructions without showing and saving each
% individual image (much faster overall), set to '0'.
show_and_save = 1;
```

After you fill out and check each of these parameters, run this section to load the variables into the Workspace. Next run Section #2, which processes all the datasets in parallel. Do not edit anything in Section #2. All images and measurements will be saved into each data folder (for setup help, please see accompanying setup documentation included in the 3DMembraneReconstruction download on GitHub: <https://github.com/mochrielab/3DMembraneReconstruction>).

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Highlights

3D membrane reconstruction allows for accurate assessment of membrane morphology and measurement of organelle volume.

The 3DMembraneReconstruction software is highly versatile and compatible with a variety of nuclear envelope markers and model organisms, including mammalian cells.

Improved facility of the software enables image optimization, batch processing, and dense sample applications (e.g., stem cells, organoids).

Fig. 1.

Choice of nuclear envelope fluorescent marker affects image contrast. (A, D) Representative wide-field fluorescence images of Nup82-GFP (A) and Heh1-GFP (D) expressing cells to assess general background fluorescence. (B, E) Zoomed-in view of the nucleus to be reconstructed (red box in A, D), highlighting the difference in image contrast (resolution) between the two markers. (C, F) Pixel intensity probability as a function of pixel intensity value for each zoomed-in image of the nucleus. The Nup82-GFP nucleus (C) exhibits a clear boundary between the signal (which we ascribe to pixels with intensities that are between one and three standard deviations above the mean pixel intensity of the entire image and are delimited by the red vertical lines in C, F and red-colored pixels in B, E). The resulting ratio between the signal and the background fluorescence, or signal-to-noise ratio (SNR) is high (3.2). By contrast, the Heh1-GFP nucleus (F) exhibits elevated background fluorescence that obscures the signal intensity resulting in a low SNR (0.9). Scale bars: (A, D) 16 μm ; (B, E) 1 μm .

Fig. 2.

Low-contrast images decrease reconstruction accuracy. (A) Fluorescent signal intensities at the nuclear membrane surface for each surface position for a Heh1-GFP (green), Nup82-GFP (blue), and Cut11-GFP (black) nucleus. Contour intensity is normalized by the mean image fluorescence intensity and shown with its mean (red dashed line) and standard deviation (shaded region). The signal intensity of Heh1-GFP at the nuclear contour with respect to its mean image fluorescence is low as compared to the normalized contour signals of Nup82-GFP and Cut11-GFP. (B) Normalized bending energies used for each surface position. Red dashed lines mark the maximum bending energy used in the reconstruction. Lower total contour signal intensity results in higher bending energies used (A, B: Heh1-GFP; green). Higher bending energy indicates an over-suppression of image detail and will diminish the reconstruction resolution.

Fig. 3.

Choice of fluorescent nuclear marker impacts the overall resolution of the reconstruction software. (A, E) Z-stack images of the fluorescently-labeled nucleus with the reconstructed nuclear envelope traced (blue line). (B, F) Nuclear cross-sectional area at each z-slice as defined by the reconstruction. The measured areas (blue circles) correspond to the blue outlines in (A, E) and are compared to the best fit to a parabola (red line), which represents the cross-sectional area of a perfect sphere. (C, G) Corresponding normalized signal intensities and bending energies used at each point of the contour surface. (D, H) 3D surface

reconstruction highlights the surface contour detail. (A) *S. cerevisiae* nucleus tagged with Nup82-GFP is able to be traced throughout the z-stack; an accurate contour fitting and 3D membrane reconstruction is achieved (B, D). (E) Conversely, Heh1-GFP demonstrates increased noise due to its relative lack of abundance at the nuclear envelope. The nuclear envelope is insufficiently detected by the reconstruction software, and 3D surface resolution is lost (F, H). Scale bars: 2 μm .

Fig. 4. Nuclear reconstruction can be achieved with an endoplasmic reticulum marker. (A) Transmitted light (left) and fluorescence (right) micrographs of *S. cerevisiae* expressing Hmg1-mCherry. (B) Nuclear cross-sectional area at each z-slice as defined by the reconstruction. The measured areas (blue circles) correspond to the blue outlines in (D) and show deviation from a perfect sphere (red parabola). (C) 3D surface reconstruction highlights the surface contour detail. (D) Z-stack images of the Hmg1-mCherry-labeled nucleus with the reconstructed nuclear envelope (blue line). Scale bars: (A) 1.6 μm ; (D) 2 μm .

Fig. 5.

To optimize temporal resolution in a microscopy time-series, z-stacks acquired with larger spatial intervals may still be used to accurately reconstruct nuclei. (A) Fluorescence micrograph of an *S. pombe* nucleus expressing Cut11-GFP. (B) Nuclear cross-sectional area at each z-slice as defined by the reconstruction. The measured areas (blue circles) correspond to the blue outlines in (D) and show deviation from a perfect sphere (red parabola). With only ten z-slices of 0.4 μm , a faithful 3D reconstruction is achieved (C). (D) Z-stack images of the Cut11-labeled nucleus with the reconstructed nuclear envelope (blue line). Scale bars: (A) 3 μm ; (E) 2 μm .

Fig. 6.

Lower-bit images lack spatial intensity information but can be adjusted to be used with the reconstruction software. (A) A 16-bit image of an *S. pombe* nucleus tagged with Cut11-GFP yields more contour resolution after image filtering to reduce background fluorescence (B) and canny-edge/fill/erosion detection of nuclear membrane (C). (D) A raw 8-bit image lacks sufficient pixel resolution to differentiate the fluorescent contour from the background after filtering and canny-edge/fill/erosion detection (E, F). (G) Histogram of 8-bit pixel intensity showcases its poor intensity range. After image adjustment to increase contrast, the pixel intensity range is enhanced (H), resulting in a contour signal that can be filtered, resolved, and eventually reconstructed (I). Scale bars: (A) 3 μm ; (D) 5 μm .

Fig. 7.

Nuclei in crowded environments may be pre-processed for reconstruction using a simple boundary exclusion regime. (A) Mouse induced pluripotent stem cells (iPS) form large colonies of closely-packed, overlapping nuclei. A single nucleus (red) has neighboring nuclear membrane that is included in the image processing (B) and is incorrectly incorporated into the surface reconstruction (C). The processed image may be manually filtered to exclude unwanted regions (D), which leaves the desired nuclear contour free of peripheral interference (E).

Fig. 8. 3D membrane reconstructions of mouse induced pluripotent stem (iPS) cells demonstrates the versatility of the reconstruction software across shape and scale. (A) A nucleus of an iPS cell that exhibits a smooth, rounded surface. (B) A nucleus from an iPS cell that has a significantly irregular nuclear surface. Scale bars: 5 μ m.

Table 1

	Live cell imaging	Fixed cell imaging
<i>S. cerevisiae</i>	Nup82-GFP	
<i>S. pombe</i>	Cut11-GFP	
Mammalian cells	GFP-mini-Nesprin2G [24]	mAb414

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