N-SIM for Quantitative Ultra-Structural Analyses of the Nuclear Lamina

Super-resolution Structured Illumination Microscopy (SIM) enables the characterization of cellular structures with approximately twice the resolution of diffraction-limited optical techniques[1], such as confocal microscopy. In this application note we highlight the work of Shimi *et al.*[2], who have applied quantitative multicolor 3D-SIM imaging using Nikon's N-SIM system towards mapping and comparing the distribution of lamin isoforms in the nuclear lamina.

Using N-SIM to characterize the distribution of lamins in the nuclear lamina

The nuclear lamins are Class V intermediate filaments that form the scaffold of the nuclear lamina, providing support and structure to the nuclear envelope. There are four principle isoforms, all of which were evaluated by Shimi *et al.*[2]: Lamin A (LA), Lamin C (LC), Lamin B1 (LB1), and Lamin B2 (LB2). Lamins play important roles in genome organization, as well as in maintaining nuclear shape and positioning. Mutations (primarily in the LMNA gene) are implicated in a variety of genetic disorders, collectively referred to as laminopathies. *In vitro*, lamin proteins form coiled-coil dimers, which then associate in a staggered head-to-tail tetramer forming the basis of the protofilament, and eventually aggregating into a paracrystalline array. However, much remains unknown about lamin organization in intact cells. In their work Shimi *et al.*[2] seek to elucidate lamin organization in mouse embryonic fibroblast (MEF) nuclei using multicolor 3D-SIM imaging with the Nikon N-SIM system.

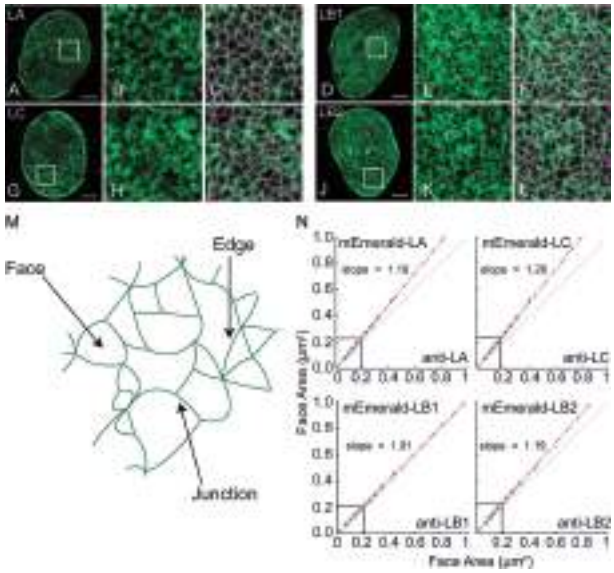

Figure 1. Using N-SIM and computational analysis to image and delineate lamin meshworks. (**a**) 3D-SIM image of mEmerald-Lamin A with zoomed-in image of white box in (**b**). (**c**) Computationally derived meshwork shown in magenta and overlaid with (**b**). (**d**) 3D-SIM images of mEmerald-Lamin B1 with zoomed (**e**) and meshwork (**f**). (**g**) 3D-SIM images of mEmerald-Lamin C with zoomed (**h**) and meshwork (**i**). (**j**) 3D-SIM images of mEmerald-Lamin B2 with zoomed (**k**) and meshwork (**l**). (**m**) Illustration of representative meshwork matrix defining meshwork face, edge, and junction. (**n**) Comparative quantile-quantile (q-q) plots comparing the face area of nuclei labeled with mEmerald fusions (y-axis) and those labeled using immunofluorescence of the same lamin isoforms (x-axis). Face area mostly skews to larger values for the FP fusions compared to respective antibody labeling (with the exception of LB1). Figure recreated with permission from ref. 2.

In order to quantify the distribution of each lamin isoform in its respective 'meshwork', computational analysis was used to perform image segmentation on the 3D-SIM slice image corresponding to the basal portion of the nuclear lamina (**Fig. 1c, f, i, l**). A steerable line filter was applied to reveal and enhance line signals, with subsequent thresholding to identify the centers of lines, and cleaned up using nonmaximal signal suppression. Gaps were then closed and the meshwork audited for oversegmentation using intensity-based quality control measures, yielding the finished product. Meshworks have several important quantifiable features: the face area (area of enclosed sections), edge length, junctions (where two or more edges meet), circularity, and more (**Fig. 1m**). N-SIM provides the resolution necessary to identify and evaluate lamin meshworks.

John R. Allen

Applications and Marketing Specialist, Nikon Instruments Inc.
1300 Walt Whitman Road, Melville NY 11747-3064, USA

Using multicolor SIM to compare protein co-distributions in macromolecular assemblies

Shimi et al.2 recognize that a significant unsolved problem in determining the macromolecular structure of the nuclear lamina is the organization of the lamins. Specifically, whether or not isoforms form distinct polymer networks and, if not, to what degree the different meshworks interact. Two-color SIM imaging (**Fig. 2**) reveals little overlap of lamin isoforms in pairwise imaging combinations. Though forming qualitatively similar meshworks, different isoforms tend to be spatially distinct from one another, with little overlap. However, shRNA-mediated silencing experiments reveal the interdependence of isoforms in meshwork morphology. Significantly, 106/226 nuclei of *Lmnb1*$^{-/-}$ MEFs demonstrated obvious enlargement of LA/C meshwork faces, an increase in face size of 34% compared to wild type. Similar results were found in other knockout MEFs, but notably less so with *Lmnb2*$^{-/-}$ MEFs.

Conclusions

Nikon's N-SIM system is a powerful platform for multi-dimensional super-resolution imaging of intact and living cells. Multi-protein macromolecular assemblies in intact cells, such as the nuclear lamina, are uniquely suited for super-resolution analyses and with greater molecular specificity than electron microscopy. Furthermore, Nikon's new N-SIM E platform makes super-resolution SIM imaging easier and more accessible than ever, and at approximately half the cost of the N-SIM. Characterization of lamin meshworks (and similar applications) could also potentially benefit from super-resolution STORM imaging. SIM typically yields about 90-120 nm lateral resolution, compared to 10-40 nm with STORM. Additionally,

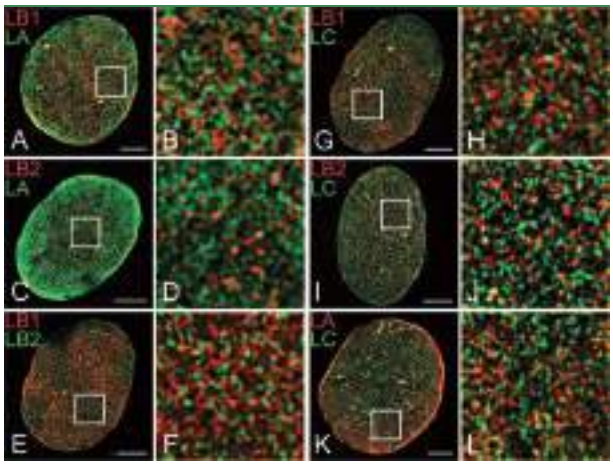

Figure 2. 2-color 3D-SIM slice images of each pair of lamin isoforms in MEFs. (**a**) LB1 (red) and LA (green) zoomed (**b**). (**c**) LB2 (red) and LA (green) zoomed (**d**). (**e**) LB1 (red) and LB2 (green) zoomed (**f**). (**g**) LB1 (red) and LC (green) zoomed (h). (**i**) LB2 (red) and LC (green) zoomed (j). LA (red) and LC (green) zoomed (**l**). Note the relatively low degree of colocalization of different isoforms. Scale bar is equal to 5 µm. Figure recreated with permission from ref. 2.

NIS-Elements features a number of integrated analysis features for performing image segmentation. To learn more about Nikon's super-resolution solutions and how they can be applied to your research, please visit our website (www.nikoninstruments.com/sr).

1. Gustafsson, M.G.L. Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy. *J. Microsc.* **198**, 82-87 (2000).

2. Shimi, T., et al. Structural organization of nuclear lamins A, C, B1, and B2 revealed by superresolution microscopy. *Mol. Biol. Cell.* **26**, 4075-4086 (2015).