

2D MAPPING OF STRONGLY DEFORMABLE CELL NUCLEI BASED ON CONTOUR MATCHING

Jonas De Vylder^{1*}, Winnok H. De Vos², Erik M. Manders^{2,3}, Wilfried Philips¹

¹ Department of Telecommunications and Information Processing, IBBT, Image Processing and Interpretation, Ghent University, St-Pietersnieuwstraat 41, 9000 Ghent, Belgium

² Department of Molecular Biotechnology, Faculty of Bioscience engineering, Ghent University, Coupure links 653, 9000 Ghent, Belgium

³ Centre for Advanced Microscopy, Section of Molecular Cytology, University of Amsterdam, Kruislaan 316, NL-1098 SM Amsterdam, The Netherlands

*Author for correspondence:

Telecommunications and Information Processing, IBBT, Image Processing and Interpretation Group, Ghent University

Tel.: 0032(0)92643416

Fax: 0032(0)92644295

E-mail: jonas.devylde@telin.ugent.be

1 Abstract

2 The spatiotemporal dynamics of protein complexes and genome loci are functionally linked to
3 cellular health status. In order to study the inherent motion of subnuclear particles, it is
4 essential to remove any superimposed component stemming from displacement and
5 deformation of the nucleus. Several models have been proposed to cope with transformations
6 occurring during basal pan-nuclear dynamics of healthy cells. However, in pathological cells,
7 such as lamin A deficient fibroblasts, the nucleus deforms in a dramatic and apparently
8 random way, making the use of such models a non trivial task. In this paper we propose a
9 mapping of the nuclear interior, which is based on the deformation of the nucleus. The
10 proposed method does not put constraints on the possible shapes or deformations. The
11 primary goal of this registration procedure is to enable an accurate estimation of telomere
12 mobility in living human cells undergoing dramatic nuclear deformations.

13

14

15

16

17

18

19

20

21

22 Key Terms

23 Image Registration, Contour Matching, Shape Normalization, Nuclear dynamics

1 **Introduction**

2 The internal organisation of the human cell nucleus in space and time is essential for its
3 function. Within the limited space of the nucleus the entire genome as well as many proteins
4 are accommodated in a non-random manner. Of particular interest are telomeres, the very
5 ends of chromosomes, which show a spatiotemporal behaviour that is functionally linked to
6 cellular and organismal condition. Telomeres are arranged in distinct patterns and display
7 mobility regimes at different time scales (1,2). Diverse biological processes such as telomere
8 maintenance, senescence and apoptosis are associated with characteristic telomere
9 redistributions (3,4). Likewise, altered telomere dynamics are associated with specific
10 diseases such as laminopathies and cancer (5,6). Therefore, quantitative studies of telomere
11 dynamics – and by extension nuclear dynamics - may help reveal novel mechanisms of
12 dysfunction and disease. However, mobility analyses in four-dimensional image data sets are
13 seriously hampered by global cell motion and nuclear deformation. This superimposes a
14 motion on the submicron dynamics, which in turn, creates a significant bias on the accuracy
15 of the measurements. It is therefore imperative to remove the superfluous motion by means of
16 image registration. A wide range of registration techniques has been proposed to remove 2D
17 and 3D motion of cell nuclei using rigid transformations, i.e. translation and rotation (7-9).
18 However, human cells often display global and local expansions or contractions, which
19 cannot be adequately modelled by rigid transformations. To cover for this biological
20 variation, a mapping based on a spherical model has been proposed (10), which captures most
21 of the cellular and nuclear displacements that occur in normal cells during interphase.
22 However, under certain pathological conditions, such as cancer and laminopathies, the nuclear
23 shape alters dramatically (11), creating a situation in which a spherical model can no longer
24 be applied (Suppl. Movie S1). Hence, a method is required that allows reliable motion
25 measurements of subnuclear features in a deformable volume without imposing any shape

1 constraints. To cater for complete non-rigid transformations, landmark-based methods have
2 been proposed (12,13), but the results of these methods are completely dependent on the
3 choice of robust landmarks, which is not always straightforward.

4 In this paper, we propose a novel approach, which maps two nuclei between consecutive time
5 points without imposing constraints (e.g. shape), nor requiring any landmarks. Instead,
6 contours are extracted from segmented nuclei, which are matched in order to achieve a point-
7 to-point correspondence. Based on this matching, a mapping of the full nucleus is calculated
8 using polyharmonic splines. This mapping predicts the location of nuclear loci, solely based
9 on the deformation of the nucleus, i.e. in the absence of individual motion. The actual
10 displacements can then be determined by comparing the predicted location with the actual
11 location of the nuclear loci of interest. The algorithm was tested both on simulated images
12 and on recordings of human cells undergoing artificially induced as well as naturally
13 occurring deformation. Using this algorithm, the overall accuracy was improved significantly
14 compared to rigid registration or state of the art Demons method (14). In addition, the
15 algorithm significantly increased the tracking efficiency by dramatically reducing the number
16 of false matches in the nearest neighbour based tracking algorithm.

17

1 **Materials and Methods**

2 *Cell culture and transfection*

3 ECV-TRF2 were cultured in advanced DMEM (Gibco, Invitrogen, USA) supplemented with
4 2% FCS and 1% penicillin/streptomycin/glutamin. ECV-TRF2, is a derivative clone from
5 ECV304 – a cell line originally described as an immortalized endothelial cell line (15) –
6 stably expressing the telomere binding fusion protein mCitrine-hTRF2 (16). Fibroblast cells
7 from a patient with a nonsense Y259X homozygous mutation in lamin A/C gene ($LMNA^{-/-}$)
8 which are completely devoid of functional lamin A/C (17), were cultured in advanced
9 DMEM/F12 (Gibco, Invitrogen, Merelbeke, Belgium) supplemented with 10% FCS and 1%
10 penicillin/streptomycin/glutamin.

11 For transfections and imaging, cells were grown on collagen coated glass bottom dishes
12 (Matek, USA). $LMNA^{-/-}$ cells were transiently transfected with the construct pmCitrine-
13 hTRF2 and Lipofectamin 2000 (Invitrogen, Merelbeke, Belgium) according to manufacturers'
14 instructions. Transiently transfected cells were imaged 24h after transfection.

15

16 *Image acquisition*

17 For live cell imaging, glass bottom dishes were filled with pre-warmed medium and sealed off
18 with parafilm, after which they were inserted into an incubator microscope and allowed to
19 equilibrate to 37°C for 30 minutes. Cells were observed under a Nikon A1r confocal
20 microscope mounted on a Nikon Ti body (Nikon Instruments, France). Experiments were
21 carried out with a Nikon Plan Apo 40x/1.3 objective. Two-hour time-lapse recordings of
22 $LMNA^{-/-}$ cells were made at 2 min intervals with minimally invasive CLEM technology (18).
23 For the validation experiments, ECV-TRF2 cells were chemically fixed by addition of cold
24 (4°C) 100% methanol (Sigma, Belgium) in fluxo, i.e. during acquisition at 30 sec intervals.

25

1 *Image Processing*

2 We have developed a novel method for full non-rigid registration of nuclei in raw CLEM
 3 images. All processing work was performed in Matlab R20007b[®] on an Intel i7 1.6GHz
 4 processor with 4 GB memory. A workflow was created of which the major aspects are
 5 described in more detail below and which are schematically summarized in Fig. 1.

6

7 *I. Segmentation*

8 Since the proposed method estimates the topological changes based on the deformation of the
 9 nucleus, the nucleus needs to be segmented first (Fig. S1). To this end, an average projection
 10 is made of the confocal 3D stack, followed by a low pass filter to reduce noise. The resulting
 11 image is then thresholded using Otsu's thresholding method, which calculates a threshold by
 12 minimizing the variance of both the foreground and background (19). Due to remaining noise,
 13 not all nucleus pixels are correctly classified as foreground; also some background pixels are
 14 wrongfully considered to be foreground. This is corrected with a morphological opening and
 15 closing. Finally the contour delineating the segment is parameterised and filtered with a
 16 Gaussian kernel, in order to obtain a smooth contour. This is important for the subsequent
 17 signature matching (see next paragraph). For more detailed work on cell nuclei segmentation,
 18 we refer to (20-22).

19

20 *II. Contour Matching*

21 Next, we find the correspondence between contours delineating the nucleus in consecutive
 22 time frames: points belonging to the contour in one frame will be linked to the corresponding
 23 points on the contour in the next frame. In order to do so, we first calculate a signature of the
 24 contour. This is a function describing the contour in one dimension. The signature is then
 25 used for the actual matching. The contour is described as $\mathbf{c}(t) = (x(t), y(t))$, with $t \in \mathbb{Z}$ and

1 $\mathbf{c}(N+1) = \mathbf{c}(1)$, i.e. the contour is periodical. Four different signatures were calculated based
 2 on $\mathbf{c}(t)$: a complex signature, a normalized centroid distance signature, a tangent direction
 3 signature and a curvature signature (Fig. S2). For a more detailed description of these
 4 signatures, see the appendix. The matching criterion we applied for two signatures is the
 5 Dynamic Time Warping (DTW) or dog-man distance. The DTW distance is the smallest
 6 possible cumulative difference between both signatures, which is found by changing the
 7 scanning speed along the contour (signature) (Fig. 1c), i.e.

$$8 \quad d_{DTW}(s_1(\cdot), s_2(\cdot)) = \min_{\gamma} \sum_t \|s_1(t) - s_2(\gamma(t))\| \quad (1),$$

9 where $\gamma(\cdot)$ is a warping function, i.e. a monotonic function mapping of $[1, N]$ onto $[1, N]$.
 10 For contours defined on a discrete grid, this can be calculated in an efficient way using
 11 dynamic programming:

$$12 \quad D(i, j) = d(i, j) + \min(\alpha D(i, j-1), \alpha D(i-1, j), D(i-1, j-1)) \quad (2)$$

13 With $d(i, j) = \|s_1(i) - s_2(j)\|$ and α a real number greater than or equal to one. In order to
 14 penalize warping functions where too many points of one signal are matched to a single point
 15 in the other signal, one can set $\alpha > 1$, for $\alpha = 1$, all warping functions are considered equally
 16 good. For the purpose of matching nuclei contours in our datasets, α was set to 1.25. The
 17 DTW distance is uniquely defined between two finite time series. On the other hand, the
 18 signatures are infinitely long, be it periodical, which makes that the signature between 0 and
 19 N can be considered finite in time. This, however, assumes that the starting point of the
 20 contour corresponds to the starting point in the other contour. If this is not the case, a wrong
 21 DTW distance and a wrong optimal warping function will be obtained. This potential pitfall is
 22 avoided by calculating the DTW distance twice: once to find the offset and a second time to
 23 find exact matching. The first time the DTW is calculated, generally results in a warping
 24 function where the start point of a signal is matched to multiple points on the other signal
 25 (Fig. 1d). If the starting point is matched to k different points, the signal is circular shifted k

1 places such that the starting point corresponds to exactly a single point. The DTW distance is
 2 now calculated for a second time, but now between the first signal and the shifted version of
 3 the second signal. The DTW distance itself is of little importance, but the warping function,
 4 $\gamma(\cdot)$ resulting in the minimal distance, is. This warping function gives the correspondence
 5 between points from both signatures. It may happen that multiple points on one contour match
 6 a single point on the second contour. This might lead to unstable solutions in the cell-mapping
 7 algorithm (see next subsection). To avoid this situation both contours are resampled. Consider
 8 the case, where one point, $s(t)$ of the signature is matched with multiple points on the other
 9 signature (cfr. Fig. 1e). First define $s'(t-1)$ and $s'(t+1)$ as the last predecessor resp. the
 10 first successor of $s(t)$ with a unique point-to-point correspondence, e.g. $s'(t-1)$ and
 11 $s'(t+1)$ respectively. The curve between $s'(t-1)$ and $s'(t+1)$ will be resampled in such
 12 a way that there are as many samples as there are samples between $s(t-1)$ and $s(t+1)$,
 13 where the samples are calculated such that the arclength between the samples is proportional
 14 to the arclength of their corresponding samples on the other signal (i.e. time point). In the
 15 example shown in Fig. 1e, this results in the replacement of $s(t)$ by two new samples, shown
 16 as black dots in the figure.

17

18 *III. Cell Mapping*

19 Based on the contour matching, each point on the contour will now correspond to a unique
 20 point on the contour of the subsequent frame. For mapping of points inside of the nucleus,
 21 $\mathbf{p} = (x, y)$, we implement polyharmonic spline interpolation:

$$22 \quad M(\mathbf{p}) = a + a_x x + a_y y + \sum_{j=1}^N \mathbf{w}_j \phi(d(\mathbf{p} - \mathbf{c}(j))) \quad (3)$$

23 where a, a_x, a_y and \mathbf{w}_j are a set of weighting coefficients, $\mathbf{c}(j)$ are the contour points, d is a
 24 distance function and $\phi(\cdot)$ is a radial basis function. We propose the commonly used

1 polyharmonic splines as radial basis functions, i.e.

$$2 \quad \phi(r) = \begin{cases} r^k, & \text{if } k \text{ is odd} \\ r^k \ln r, & \text{if } k \text{ is even} \end{cases} \quad (4),$$

3 where k is the degree of the basis functions. The weighting coefficients in eq. (3) are
4 calculated by solving a system of linear equations:

5 • N equations that map the points on the contour to their matching points on the other
6 contour, i.e. $M(\mathbf{c}(t)) = \mathbf{c}'(t)$ where $\mathbf{c}'(t)$ is the matching contour point of $\mathbf{c}(t)$.

7 • two equations imposing orthogonality of the weighting coefficients, \mathbf{w} onto the basis

8 function, i.e. $\sum_{i=1}^N \mathbf{w}_i x_i = \sum_{i=1}^N \mathbf{w}_i y_i = 0$.

9 • one equation forcing the weighting coefficients to sum to zero: $\sum_{i=1}^N \mathbf{w}_i = 0$

10 This system can be solved using any solver for linear systems, e.g. Gaussian elimination. For
11 a fast solver for this specific linear system we refer to (25). A more detailed discussion on
12 polyharmonic splines and the influence of the different radial basis functions can be found in
13 (26).

14 Although polyharmonic interpolation has been proven useful for non-rigid registration, some
15 adjustments are needed in order to use them in this context. The mapping of a point in the
16 nucleus depends on the Euclidean distance between the point itself and the contour points.
17 This distance however does not take the nuclear shape into account. By ignoring the nuclear
18 shape we overestimate the motion of contour points which might be close by in the image, but
19 which can be far away in the nucleus (Fig. S3). Instead we propose using the geodesic
20 distance. The length of the shortest path not crossing the cell wall is considered to be the
21 geodesic distance between A and B. The geodesic distance was calculated using the fast
22 marching toolbox from Peyre (28)

1 A second adjustment concerns the location of the maximal displacement: We assume that the
2 displacement is maximal along the contour of the mapping. Hence, to avoid the maximum
3 displacement from falling inside the nucleus, contour points were added at a short distance
4 outside of the nucleus, causing the derivatives of the mapping normal to the contour to equal
5 zero (Fig S4).

6

7 *IV. Telomere Tracking*

8 In order to study telomere dynamics over time, detected telomeres in consecutive time frames
9 are matched using the Iterative Closest Point method (ICP). This results in a matching where
10 each telomere is matched with exactly one telomere in the next frame. ICP is a greedy
11 algorithm, i.e. the closest telomere points are matched first and this decision is not revised in
12 the remaining of the algorithm. This is iteratively repeated until all telomeres are matched.
13 Manual annotation of tracks and images was performed in ImageJ freeware
14 (<http://imagej.nih.gov/ij/>) expanded with the MTrackJ plugin (by E.Meijering,
15 <http://www.imagescience.org/meijering/>).

16

17

1 **Results**

2 First, the mapping algorithm was validated on an artificial sequence using a Monte Carlo
3 simulation. The nucleus was approximated by a flattened ellipsoid (80x50x40voxels) in
4 which 60 random points were generated, representing telomere positions. Then the ellipsoid
5 was deformed using a random affine transformation composed of 3 separate components: the
6 three axes of the ellipsoid were independently rescaled (between a factor 0.9 and 1.1), the
7 ellipsoid was rotated (between -5° and $+5^\circ$ along the long axis of the lateral plane and
8 between -10° and $+10^\circ$ along the axial axis) and translated (between -10 and +10 pixels in the
9 lateral direction). 10000 simulations were performed resulting in average telomere
10 displacement of 8.7 ± 15.7 pixels. Subsequent contour matching and point tracking resulted on
11 average (all transformations combined) in a remaining telomere motion of 2.4 ± 3.5 pixels (or
12 72.5% reduction), only coming from the motion and deformation of the nucleus. This Monte
13 Carlo simulation was repeated with only transformations in the lateral plane, i.e. without the
14 rotation around the ellipsoids axis and without the scaling in the non-lateral direction. The
15 simulation of only lateral transformations resulted in an average remaining telomere motion
16 of 2.2 ± 3.7 pixels (74% reduction).

17 As it is not known beforehand what the actual mobility of objects in a nucleus will be, it is
18 difficult to validate the algorithm on a real dataset per se. To test the accuracy of the
19 algorithm we diverted to a dataset with maximal resemblance to the natural situation: ECV-
20 TRF2 cells were acquired during fixation with methanol (Fig. 2). From the moment methanol
21 is added, all active and intrinsic motion is blocked. At the same time, the cold methanol
22 induces a contraction of the entire cell, including the nucleus, which persists for about 10 to
23 20 min. In this setting, telomeres only become displaced by the deformation of the nucleus
24 and the telomeres' centres can therefore serve as ground truth for validation, i.e. the
25 performance of the algorithm can be judged by its' relative minimization of the displacement

1 error. Although the induced shrinking is relatively isotropic, not all nuclei deform in the same
2 way, not all parts of the nucleus contract equally and not all telomeres relocate equally far. 97
3 telomeres coming from 3 different nuclei were manually tracked during 7 frames. This
4 resulted in 582 ground true telomere matches. The influence of the different signatures as well
5 as the different radial basis functions used by the mapping was tested. These tests were
6 performed using 60 sample points for the contours; more samples did not yield better results
7 (Fig. S5).

8 The proposed mapping was compared with a rigid mapping and with a non-rigid mapping
9 based on the Demons registration algorithm (27). The following metrics were used for error
10 estimation: remaining telomere motion, standard deviation of the motion and the number of
11 false matches in the tracking algorithm (Table 1). A linear basis, i.e. polyharmonic basis of
12 degree 1, performed best for all four signatures. From the four signatures tested, the "tangent
13 direction signature" gave the best result.

14 Finally, the algorithm was applied to a real dataset from transiently transfected LMNA^{-/-} cells.
15 Nuclei from these cells display dramatic, apparently random deformations, which clearly
16 drive a subset of telomeres in a given direction. This makes it difficult to estimate the inherent
17 mobility of the telomeres within the nucleus. 34 telomeres coming from 3 different nuclei
18 where manually tracked over 9 frames. This results in ground truth of 272 telomere
19 displacements. In Fig. 3g an example of the mapping is shown. As can be seen, the mapping
20 removes most of the motion stemming from nuclear deformation (Table 2). These results
21 show a significant difference over non-processed data. Again, these results confirm the
22 biharmonic radial basis and directional signature as the proposed mapping which results in the
23 least telomere displacement. Note that this displacement is not a measure of error since these
24 results come from living nuclei, i.e. there is still telomere motion coming from other
25 biological processes than nuclear deformation. This telomere motion should however be

1 considerable smaller than the motion induced due to nuclear deformation and displacement.
2 It took on average 2689 ms to calculate the weighting parameters used by the mapping, i.e.
3 \mathbf{w}_j , a , a_x and a_y in eq.(3). For each telomere, the calculation of its mapped location took an
4 additional 103 ms. Approximately 90% of this time was spent on the calculation of the
5 geodesic distance between points. There is no significant difference in computational time
6 depending on the type of signature or on the type of basis function used.

1 **Discussion**

2 In this paper, a new framework was proposed for registering cell nuclei without any shape
3 constraints. The proposed technique works in two steps. First, nuclear boundaries are
4 optimally aligned through time, by matching their contour signatures. Of the four different
5 contour signatures that were tested, the tangent direction signature proved most efficient.
6 Based on this contour matching, the entire nucleus is mapped using polyharmonic
7 interpolation, radial basis of degree one gave the best results.

8 The primary goal of this registration procedure was to enable an accurate estimation of
9 telomere mobility in living human cells undergoing dramatic nuclear deformations such as
10 human LMNA^{-/-} cells. The nuclei in the 4D CLEM recordings display both arbitrary nuclear
11 deformation as well as stochastic lower level telomere mobility, making it difficult to assess
12 the true performance of the algorithm on these datasets alone. On the other hand, it was
13 difficult to simulate such a multifactorial dynamic system in silico, as this would have
14 required explicit knowledge on the expected biological deformations. Therefore, we have
15 limited the possible deformations in the simulation to affine mappings. In an attempt to
16 overcome the limitations of the simulations, we devised a method for controlled deformation,
17 devoid of individual object mobility, with maximal correspondence to the actual biological
18 topic of interest, namely a chemically induced shrinking procedure. To our knowledge, this is
19 the first time such an approach was adopted for mimicking deformation of biological material
20 in an uncompromised and controlled manner. Using this approach, we demonstrated a 5.5
21 fold increase in localization accuracy with respect to the raw data set. Some part of the
22 remaining error of 64.85nm/min might be due to the contribution of axial components in 3-
23 dimensional movements, which may have been obscured by mapping the deformation in 2D
24 (on projections). However, both cell types under investigation, are adherent and have a
25 flattened nuclear shape in the axial direction suggesting that most of the deformation does

1 indeed occur in the lateral direction (28). Nevertheless, neither axial deformation, nor rolling
2 phenomena can be excluded. In order to estimate the influence of these non-lateral
3 deformations, simulations with all possible 3-dimensional deformations were compared with
4 simulations containing only lateral deformations. This resulted in a net gain of 2% removal of
5 telomere motion compared to the full deformation simulations (74% vs. 72%), which shows
6 that lateral movements indeed cause most of the motion and 2D mapping already removes a
7 major contribution. This is based on the assumption of an affine transformation, whereas
8 nuclei are obviously not restricted to this type of stringent transformations. Future work
9 should therefore focus on extending the method to 3D, e.g. by warping contours on a slice by
10 slice basis, or by using more complex 3D shape matching techniques, before applying a 3D
11 polyharmonic spline mapping. Next to the error induced by 2D mapping, both the
12 interpolation as the shape matching will introduce a small error as well as both are
13 approximations of the real biological deformation. Nevertheless, despite of these
14 approximations, the proposed method performs better than the current state of the art (27).

15 The essential novelty of our work is that, in contrast to most of the existing methods, no
16 specific shape constraints are imposed on the contour mapping (10, 12). By doing so, it is
17 possible to map contours with a non-elliptical shape, which lack clear and stable landmarks.
18 Some work has been done on generic non-rigid registration without shape constraints
19 (13,14,27), but several of these methods use intensity, which is unstable and highly variable
20 in fluorescent images (27). Yang et al. proposed a method based on the mapping of
21 segmented nuclei, but their method has difficulties coping with strong deformations, e.g. not
22 all telomeres are mapped inside the nucleus (27). The only work known to the authors
23 handling strongly deformed nuclei restricts itself to shape analysis (29), but does not result in
24 a nuclear mapping, which enables the study of telomere dynamics.

25 In live cell data it became clear that this type of registration is essential for accurate

1 estimation of the telomere displacements. The average displacement was reduced by a factor
2 2.8 with the proposed mapping: 189 ± 42 nm/min without registration, and 66 ± 11 nm/min
3 using the mapping. This obviously emphasizes the importance of the mapping for accurate
4 mobility estimations. The mapping does not only influence the quantitative measurements of
5 telomere dynamics, but also has a positive impact on the tracking algorithm. The number of
6 false matches in the iterative closest point algorithm reduces with approximately 61%, which
7 is an improvement of 12% compared to registration using the Demons method (27).

8 In conclusion, we have presented a novel registration technique for mapping strongly
9 deformable nuclei. Given to the large variety of pathologies (laminopathies, cancer...) and
10 processes (apoptosis, mitosis) that are associated with strong nuclear deformations, it can be
11 expected that this algorithm will have generic value and improve the accuracy of subnuclear
12 mobility of genome loci as well as nuclear protein bodies.

13

1 Appendix

2 A wide variety of signatures to describe shapes have been proposed in literature (23,24).

3 Within this work, the following four different signatures were tested:

4 • A complex signature: $s(t) = x(t) + jy(t)$, where $j^2 = -1$.

5 • a normalized centroid distance signature: $s(t) = \frac{\sqrt{(x(t) - x_c)^2 + (y(t) - y_c)^2}}{\sum_{i=1}^N \sqrt{(x(i) - x_c)^2 + (y(i) - y_c)^2}}$,

6 where (x_c, y_c) is the centroid of \mathbf{c} . Note that this signature is normalized such that

7 $\int s(t)dt = 1$. By doing so, this signature is scaling invariant, i.e. invariant to isotropic

8 expansion and contraction of the nucleus.

9 • A direction signature: $s(t) = \cos^{-1}\left(\frac{y(t+1) - y(t-1)}{\|\mathbf{c}(t+1) - \mathbf{c}(t-1)\|}\right)$. This signature corresponds to

10 the orientation of the tangent line at point (t).

11 • A curvature signature: $s(t) = \left\| \frac{\partial^2 \mathbf{c}(t)}{\partial t^2} \right\|$. The curvature signature has low values at places

12 where the contour is smooth and high values at places where the contour has sharp

13 angles.

14

1

2 **Acknowledgment**

3 Jonas De Vylder is funded by the Institute for the Promotion of Innovation by Science and
4 Technology in Flanders (IWT). The financial support of the Hercules Foundation (project
5 AUGE/013) is gratefully acknowledged. The department of Molecular Biotechnology is a
6 cofounder of NB-photonics, Ghent multidisciplinary partnership. We thank prof. H. Tanke,
7 University of Leiden, The Netherlands for providing the construct pmCitrine-hTRF2.

1 References

- 2 1. De Vos WH, Houben F, Hoebe RA, Hennekam R, van Engelen B, Manders EM,
3 Ramaekers FC, Broers JL, Van Oostveldt P. Increased plasticity of the nuclear
4 envelope and hypermobility of telomeres due to the loss of a-type lamins. *Biochim*
5 *Biophys Acta*.
- 6 2. Molenaar C, Wiesmeijer K, Verwoerd NP, Khazen S, Eils R, Tanke HJ, Dirks RW.
7 Visualizing telomere dynamics in living mammalian cells using PNA probes. *The*
8 *EMBO Journal* 2003;22(24):6631-6641.
- 9 3. Raz V, Carlotti F, Vermolen BJ, van der Poel E, Sloos WCR, Knaan-Shanzer S, de
10 Vries AAF, Hoeben RC, Young IT, Tanke HJ and others. Changes in lamina structure
11 are followed by spatial reorganization of heterochromatic regions in caspase-8-
12 activated human mesenchymal stem cells. *Journal of Cell Science* 2006;119(20):4247-
13 4256.
- 14 4. Raz V, Vermolen BJ, Garini Y, Onderwater JJ, Mommaas-Kienhuis MA, Koster AJ,
15 Young IT, Tanke H, Dirks RW. The nuclear lamina promotes telomere aggregation
16 and centromere peripheral localization during senescence of human mesenchymal
17 stem cells. *J.Cell Sci.* 2008;121(Pt 24):4018-4028.
- 18 5. De Vos WH, Houben F, Hoebe RA, Hennekam R, van Engelen B, Manders EM,
19 Ramaekers FC, Broers JL, Van Oostveldt P. Increased plasticity of the nuclear
20 envelope and hypermobility of telomeres due to the loss of A-type lamins. *Biochim*
21 *Biophys Acta*;1800(4):448-58.
- 22 6. Louis SF, Vermolen BJ, Garini Y, Young IT, Guffei A, Lichtensztejn Z, Kuttler F,
23 Chuang TCY, Moshir S, Mougey V and others. c-Myc induces chromosomal
24 rearrangements through telomere and chromosome remodeling in the interphase
25 nucleus. *Proceedings of the National Academy of Sciences of the United States of*

- 1 America 2005;102(27):9613-9618.
- 2 7. Wilson CA, Theriot JA. A correlation-based approach to calculate rotation and
3 translation of moving cells. *Ieee Transactions on Image Processing* 2006;15(7):1939-
4 1951.
- 5 8. Gerlich D, Beaudouin J, Gebhard M, Ellenberg J, Eils R. Four-dimensional imaging
6 and quantitative reconstruction to analyse complex spatiotemporal processes in live
7 cells. *Nature Cell Biology* 2001;3(9):852-855.
- 8 9. Matula P, Matula P, Kozubek M, Dvorak V. Fast point-based 3-D alignment of live
9 cells. *Ieee Transactions on Image Processing* 2006;15(8):2388-2396.
- 10 10. Gladilin E, Goetze S, Mateos-Langerak J, Van Driel R, Eils R, Rohr K. Shape
11 normalization of 3D cell nuclei using elastic spherical mapping. *Journal of*
12 *Microscopy-Oxford* 2008;231(1):105-114.
- 13 11. Capo-chichi CD, Cai KQ, Testa JR, Godwin AK, Xu XX. Loss of GATA6 leads to
14 nuclear deformation and aneuploidy in ovarian cancer. *Mol Cell Biol*
15 2009;29(17):4766-77.
- 16 12. Mattes J, Nawroth J, Boukamp P, Eils R, Greulich-Bode KM. Analyzing motion and
17 deformation of the cell nucleus for studying co-localizations of nuclear structures.
18 2006 3rd IEEE International Symposium on Biomedical Imaging: Macro to Nano,
19 Vols 1-3 2006:1044-1047.
- 20 13. Zitova B, Flusser J. Image registration methods: a survey. *Image and Vision*
21 *Computing* 2003;21(11):977-1000.
- 22 14. Tiehua Du MW. 3D Image Stack Reconstruction in Live Cell Microscopy of
23 *Drosophila* Muscles and its Validation. *Cytometry A* 2009;75:329-343.
- 24 15. Takahashi K, Sawasaki Y, Hata J, Mukai K, Goto T. Spontaneous transformation and
25 immortalization of human endothelial cells. *In Vitro Cell Dev.Biol.* 1990;26(3 Pt

- 1 1):265-274.
- 2 16. De Vos WH, Hoebe RA, Joss GH, Haffmans W, Baatout S, Van Oostveldt P, Manders
3 EM. Controlled light exposure microscopy reveals dynamic telomere microterritories
4 throughout the cell cycle. *Cytometry A* 2009;75(5):428-39.
- 5 17. Muchir A, van Engelen BG, Lammens M, Mislou JM, McNally E, Schwartz K,
6 Bonne G. Nuclear envelope alterations in fibroblasts from LGMD1B patients carrying
7 nonsense Y259X heterozygous or homozygous mutation in lamin A/C gene. *Exp.Cell*
8 *Res.* 2003;291(2):352-362.
- 9 18. Hoebe RA, Van Oven CH, Gadella TW, Jr., Dhonukshe PB, Van Noorden CJ,
10 Manders EM. Controlled light-exposure microscopy reduces photobleaching and
11 phototoxicity in fluorescence live-cell imaging 1. *Nat.Biotechnol.* 2007;25(2):249-
12 253.
- 13 19. Otsu N. Threshold Selection Method from Gray-Level Histograms. *IEEE Transactions*
14 *on Systems Man and Cybernetics* 1979;9(1):62-66.
- 15 20. Cloppet F, Boucher A. Segmentation of overlapping/aggregating nuclei cells in
16 biological images. 19th International Conference on Pattern Recognition, Vols 1-6
17 2008:789-792.
- 18 21. Yan PK, Zhou XB, Shah M, Wong STC. Automatic segmentation of high-throughput
19 RNAi fluorescent cellular images. *IEEE Transactions on Information Technology in*
20 *Biomedicine* 2008;12(1):109-117.
- 21 22. Li G, Liu T, Nie J, Guo L, Chen J, Zhu J, Xia W, Mara A, Holley S, Wong STC.
22 Segmentation of touching cell nuclei using gradient flow tracking. *Journal of*
23 *Microscopy-Oxford* 2008;231(1):47-58.
- 24 23. Kindratenko VV. On using functions to describe the shape. *Journal of Mathematical*
25 *Imaging and Vision* 2003;18(3):225-245.

- 1 24. Zhang DS, Lu GJ. Review of shape representation and description techniques. Pattern
2 Recognition 2004;37(1):1-19.
- 3 25. Beatson RK, Powell MJD, Tan AM. Fast evaluation of polyharmonic splines in three
4 dimensions. IMA Journal of Numerical Analysis 2007;27(3):427-450.
- 5 26. Arad N, Dyn, Nira, Reisfeld, Daniel, Yeshurun, Yehezkel. Image warping by radial
6 basis functions: applications to facial expressions. CVGIP: Graph. Models Image
7 Process. Volume 56; 1994. p 161-172.
- 8 27. Yang SW, Kohler D, Teller K, Cremer T, Le Baccon P, Heard E, Eils R, Rohr K.
9 Nonrigid registration of 3-D multichannel microscopy images of cell nuclei. IEEE
10 Transactions on Image Processing 2008;17(4):493-499.
- 11 28. Koopman W, Verkaart S, van Emst-de Vries S, Grefte S, Smeitink J, Willems P.
12 Simultaneous Quantification of Oxidative Stress and Cell Spreading Using 5-(and-6)-
13 Chloromethyl-20,70-dichlorofluorescein. Cytometry A 2006;69A:1184–1192.
- 14 29. Rohde GK, Ribeiro AJ, Dahl KN, Murphy RF. Deformation-based nuclear
15 morphometry: capturing nuclear shape variation in HeLa cells. Cytometry A
16 2008;73(4):341-50.
- 17 30. Peyre, G. Toolbox Fast Marching - A toolbox for Fast Marching and level sets
18 computations,
19 <http://www.ceremade.dauphine.fr/~peyre/matlab/fast-marching/content.html>
20
21

1 **Figure Legends**

2

3 Figure 1. The workflow of the proposed method: (a) The nucleus is first segmented and
 4 smooth outlines are generated (red) (b) A 1-dimensional contour signature is calculated for
 5 the segmented nucleus (c) The signatures of two consecutive frames are matched according to
 6 the dynamic time warping technique (d) The alignment matrix, $D(\dots)$, between a signal and a
 7 cyclic shifted version of the same signal is shown. The optimal warping path is represented by
 8 gray solid squares. The mismatch of the starting point corresponds with the horizontal part at
 9 the beginning of the warping path (e) The signatures are resampled such that there is a point-
 10 to-point correspondence (f) Based on the contour matching, a full nucleus mapping is
 11 calculated using polyharmonic spline interpolation.

12

13 Figure 2. Contour mapping applied to a chemically shrunken nucleus. ECV-TRF2 cells were
 14 acquired at 30sec intervals with in fluxo addition of methanol after 5min of acquisition (a-c)
 15 Fluorescent micrographs showing progressive shrinking throughout the 25min acquisition
 16 period (d) Telomere motion over a period of 15 minutes, represented as color-coded tracks of
 17 the telomeric centres of mass. The calibration ranges from 10min (white) to 25min (dark blue)
 18 (e) Deformation of the nucleus over the same period, represented by the nuclear outlines of
 19 consecutive time points (f) The nuclear mapping result after contour matching using a
 20 complex signature and a radial basis of degree 1, superimposed onto the outlines of the
 21 nucleus at 10min (red) and 25min (blue). The gradient displacement calibration bar ranges
 22 from 28nm (black) to 2990nm (white) (g) The result of telomere mapping after contour
 23 matching using a complex signature and a radial basis of degree 1. The red and blue curves
 24 are the contours of the nucleus at 10min and 25min and the red and blue dots mark the
 25 centroids of the telomeres of the respective time points. The green dots represent the location

1 of the telomeres predicted by the mapping, i.e. solely based on the deformation.

2

3 Figure 3. Contour mapping applied on naturally deforming nucleus from an LMNA^{-/-} cell,

4 transiently transfected with pmCitrine-hTRF2, acquired at 2min intervals (a-c) Fluorescent

5 micrographs at different time points. (d) Telomere motion over a period of 18 minutes,

6 represented as color-coded tracks of the telomeric centres of mass (e) Deformation of the

7 nucleus over the same period, represented by the nuclear outlines of consecutive time points

8 (f) The nuclear mapping result after contour matching using a complex signature and a radial

9 basis of degree 1, superimposed onto the outlines of the nucleus at 10min (red) and 25min

10 (blue). The gradient displacement calibration bar ranges from 259nm (black) to 2385nm

11 (white) (g) The result of telomere mapping after contour matching using a complex signature

12 and a radial basis of degree 1. Red, blue and green represent features of the first time point,

13 last time point (18min) and mapping-based prediction, respectively.

14

15

1 **Supplementary Material**

2

3 Movie S1. Composite movie illustrating dramatic nuclear deformations in LMNA^{-/-} cells.
4 Three nuclei of LMNA^{-/-} cells, which were transiently transfected with pmCitrine-hTRF2 and
5 acquired with CLEM at 2min intervals, were stitched together in a single montage. The
6 individual nuclear outlines were derived from the nuclear background signal and
7 superimposed on the time stacks for highlighting the deformation. The scale bar represents
8 10 μ m.

9

10 Figure S1: The consecutive steps of the segmentation algorithm: (a) low pass filtering of the
11 image, (b) Otsu thresholding, (c) morphological closing, (d) morphological opening (e) low-
12 pass filtering the contour.

13

14 Figure S2: Four different shape signatures corresponding with the contour of the nucleus in
15 figure S1 (a) complex signature; (b) normalized centroid distance signature; (c) tangent
16 direction signature and (d) curvature signature

17

18 Figure S3: An example of two different distance functions: the Euclidean distance on the left,
19 the geodesic distance on the right. The distance between points A and B corresponds to the
20 length of the dashed line, where the blue line represents the nucleus contour.

21

22 Figure S4: Two examples of polyharmonic spline interpolation for nucleus mapping. In (a,c)
23 the gray value at a point in the nucleus corresponds to the magnitude of the displacement
24 vector at that point. (b,d) Is a profile corresponding with the green line in respectively (a) and
25 (c). The top figures correspond to polyharmonic spline mapping with radial basis of degree

1 two. Note that the maximal displacement of the nucleus doesn't lie on the contour, which is
2 unlikely under the assumption that the interior displacement is due to a deformation of the
3 nuclear contour. The lower figures represent our proposed mapping where the displacement is
4 imposed to be maximal at the contour, also with radial basis of degree 2.

5

6 Figure S5: The influence of the number of contour samples on the accuracy of the telomere
7 mapping. Calculated on the methanol dataset, using the tangent direction signature and the
8 polyharmonic basis of degree 1.

9