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Probing compressibility of the nuclear interior in wild-type and lamin deficient cells using microscopic imaging and computational modeling

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ABSTRACT

Mechanical properties of the cell nucleus play an important role in maintaining the integrity of the genome and controlling the cellular force balance. Irregularities in these properties have been related to disruption of a variety of force-dependent processes in the cell, such as migration, division, growth or differentiation. Characterizing mechanical properties of the cell nucleus *in situ* and relating these parameters to cellular phenotypes remain challenging tasks, as conventional micromanipulation techniques do not allow direct probing of intracellular structures. Here, we present a framework based on light microscopic imaging and automated mechanical modeling that enables characterization of the compressibility of the nuclear interior *in situ*. Based entirely on optical methods, our approach does not require application of destructive or contacting techniques and it enables measurements of a significantly larger number of cells. Compressibility, in this paper represented by Poisson's ratio ν, is determined by fitting a numerical model to experimentally observed time series of microscopic images of fluorescent cell nuclei in which bleached patterns are introduced. In a proof-of-principle study, this framework was applied to estimate ν in wild type cells and cells lacking important structural proteins of the nuclear envelope ($LMNA^{-/-}$). Based on measurements of a large number of cells, our study revealed distinctive changes in compressibility of the nuclear interior between these two cell types. Our method allows an automated, contact-free estimation of mechanical properties of intracellular structures. Combined with knockdown and overexpression screens, it paves the way towards a high-throughput measurement of intracellular mechanical properties in functional phenotyping screens.

1. Introduction

The cell nucleus provides eukaryotic cells with a functional scaffold to store and protect the genome while at the same time ensuring the proper execution of essential process (Iborra and Cook, 2002). The mechanical properties of the cell nucleus maintain the integrity of the genome and contribute to the cellular force balance (Rowat et al., 2008; Dahl et al., 2010). Irregularities in these properties are related to alterations of force-dependent processes like migration, division, growth and differentiation and thus play an important role in the development of diseases like the laminopathies (Broers et al., 2004) and cancer (Kumar and Weaver, 2009).

A variety of biophysical assays coupled with *in silico* analyses have been developed to determine the mechanical properties of cells for diverse purposes and at different spatial scales (Vaziri and Gopinath, 2007). Most of these methods are based on calculation of the cellular response to application of external loads onto the cellular membrane. The extension of these techniques devoted to the study of whole cell mechanics to the biomechanical analysis of intracellular structures such as cell nuclei is not straightforward, since these structures are not accessible for direct mechanical contact in a non-destructive way. Many existing approaches to investigate nuclear mechanics rely on prior isolation of cell nuclei (Lammerding et al., 2007; Vaziri et al., 2006; Vaziri and Mofrad, 2007; Guilak et al., 2000), removing them from the physiological environment inside the cell, i.e. the physiological concentrations of salts, which has been shown to alter nuclear mechanical properties (Dahl et al., 2005). Other experimental setups require time-expensive and extensive procedures that complicate the reproducibility of experiments and limit the throughput. Recently developed optical stretchers (Guck et al., 2001, 2005) enable a contactless probing of thousands of cells. However, these methods are incapable of extracting the contribution of the nucleus on the overall cellular response, and thus cannot be applied to measure the material properties of the cell nucleus. An automated analysis of nuclear mechanics is of particular interest for medical research,

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e.g. in cancer, where routine measurements of a statistically-significant number of samples are required to account for large inhomogeneities characterizing tumor cells. To overcome shortcomings of conventional methods of experimental cell mechanics, we have been developing frameworks for image and model-based analysis of material properties of intracellular structures in living cells (Gladilin et al., 2007). Here, we extend our previous feasibility study (Gladilin et al., 2010) and describe a complete framework to determine compressibility of the nuclear interior by introducing bleached patterns on fluorescently stained chromatin. In contrast to previous works that rely on geometrical description of the overall nuclear shape and, thus, imply a one-material approximation of the entire nucleus (Caille et al., 2002) to determine an apparent Poisson's ratio (Ofek et al., 2009; Leipzig and Athanasiou, 2008), our approach is designed to probe the Poisson's ratio of the nuclear interior of which the mechanical properties are different from those of the nuclear membrane or a one-material nucleus model. We present a comparative study on determination of intranuclear compressibility in wild type and LMNA$^{-/-}$ mouse embryonic fibroblasts. LMNA$^{-/-}$ cells lack the nuclear envelope proteins lamin A and C, and their nuclei are known to be structurally and mechanically impaired (Sullivan et al., 1999). An increased fragility and reduced nuclear stiffness in comparison to their wild type counterparts have been measured (Lammerding et al., 2004). However no studies regarding the compressibility of these nuclei have been performed so far.

2. Methods

2.1. Numerical model of nuclear mechanics

Based on previous experimental observations (e.g., Thoumine and Ott, 1997), the nucleus was approximated as a homogeneous, isotropic material described by the St. Venant–Kirchhoff constitutive law (Ciarlet, 1988). In the case of the pure displacement problem, that is forces are given implicitly as the boundary displacements, the linearized material law is given by Lame–Navier partial differential equation of the displacement $\mathbf{u}$:

$$\Delta\mathbf{u}+\frac{1}{1-2\nu}\text{grad div }\mathbf{u}=0, \tag{1}$$

Eq. (1) contains only one material parameter, namely, the Poisson's ratio ν describing the material's compressibility. Consequently, the continuum displacement obtained as a solution of Eq. (1) depends on ν. To compute the displacements of the nuclear interior resulting from the prescribed displacements of the nuclear boundary, the pure-displacement finite element method (FEM) was applied (Gladilin et al., 2007).

2.2. Determining compressibility of the nuclear interior from image time series

Our approach to determine compressibility of the nuclear interior is based on two general principles:

(i) Greens's integration theorem (in application to continuum mechanics better known as the Somigliana's identity (Beskos, 1987) that establishes a unique relationship between the prescribed boundary displacements, the resulting displacements of the domain interior and the material properties of mechanical continuum;
(ii) Reformulation of a parameter estimation problem as an image registration problem, which consists in minimization of dissimilarity between the numerically simulated and experimentally observed images.

In order to incorporate these two principles in our numerical framework, two different data modalities for representation of the cell nuclei were combined. Finite element (FE) models were generated to compactly assess the 3D nuclear shape and to compute the displacements of the nuclear interior nodes from the prescribed displacements of the nuclear boundary nodes. Time series of 3D microscopic images were used to monitor temporal changes of the intranuclear structure and to evaluate the predictions of numerical simulations. The mapping of nodal displacements of the FE mesh onto 3D image coordinates was implemented using standard tetrahedral shape functions. An overview of our image and model-based framework for determination of intranuclear compressibility is in Fig. 1. The goal of this framework consists in the establishment of a unique relationship between ν and the intensity changes in microscopic images of deforming nuclei. This relationship is implicitly given by the ν-dependent displacement of the nuclear interior $\mathbf{u}(\nu)$. In our previous work (Gladilin et al., 2010), it has been shown

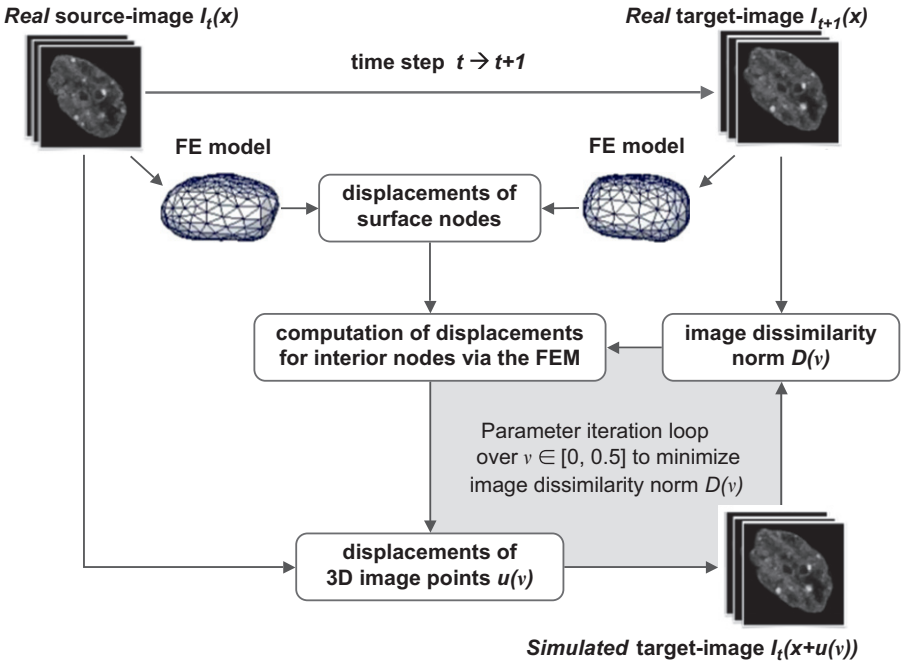

Fig. 1. Overall scheme of image- and model-based framework for determination of intranuclear compressibility. For each pair of adjacent time steps, finite element models of the nuclei are generated from 3D microscopic images. The displacements of the surface nodes are given by the surface correspondences mapping the nuclear boundary from one time step onto the next. The displacements of the interior nodes $\mathbf{u}(\nu)$ are computed from the displacements of the surface nodes via the FEM and are applied to the source-image $I_t(\mathbf{x})$ to calculate its deformed configuration $I_t(\mathbf{x}+\mathbf{u}(\nu))$, i.e., the simulated target-image. The minimum of the dissimilarity norm $\mathcal{D}(\nu_{\min})=\min(\mathcal{D}(\nu))$ between the simulated target-image $I_t(\mathbf{x}+\mathbf{u}(\nu))$ and the real next time-step image $I_{t+1}(\mathbf{x})$ corresponds to Poisson's ratio ν_{min} of the nuclear interior.