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Compressed sensing traction force microscopy

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ABSTRACT

Adherent cells exert traction forces on their substrate, and these forces play important roles in biological functions such as mechanosensing, cell differentiation and cancer invasion. The method of choice to assess these active forces is traction force microscopy (TFM). Despite recent advances, TFM remains highly sensitive to measurement noise and exhibits limited spatial resolution. To improve the resolution and noise robustness of TFM, here we adapt techniques from compressed sensing (CS) to the reconstruction of the traction field from the substrate displacement field. CS enables the recovery of sparse signals at higher resolution from lower resolution data. Focal adhesions (FAs) of adherent cells are spatially sparse implying that traction fields are also sparse. Here we show, by simulation and by experiment, that the CS approach enables circumventing the Nyquist–Shannon sampling theorem to faithfully reconstruct the traction field at a higher resolution than that of the displacement field. This allows reaching state-of-the-art resolution using only a medium magnification objective. We also find that CS improves reconstruction quality in the presence of noise.

Statement of Significance

A great scientific advance of the past decade is the recognition that physical forces determine an increasing list of biological processes. Traction force microscopy which measures the forces that cells exert on their surroundings has seen significant recent improvements, however the technique remains sensitive to measurement noise and severely limited in spatial resolution. We exploit the fact that the force fields are sparse to boost the spatial resolution and noise robustness by applying ideas from compressed sensing. The novel method allows high resolution on a larger field of view. This may in turn allow better understanding of the cell forces at the multicellular level, which are known to be important in wound healing and cancer invasion.

As the smallest units of life, cells are able to exert forces in order to fulfill a number of functions during embryonic development, tissue regeneration, wound healing, and the immune response [1–7]. A basic trait of adhering cells is their contractility when adhered to a substrate. Contractility is generated by the cytoskeleton and transmitted to the substrate via discrete focal adhesions (FAs). The resultant forces trigger activation of signaling pathways in the cell [8], tune the binding rate of adhesion molecules [9], or alter the expression of a number of genes [10]. Downstream effects of force application include the dependence of stem cell differentiation and cancer cell invasion on substrate rigidity [11,12].

Traction force microscopy (TFM) is the standard technique used to measure the forces exerted by single cells or cell colonies on relatively soft elastic substrates [13]. The method was pioneered by Dembo et al. [14], and subsequently modified to enable shorter computing time [15], finite element calculation [16,17], higher resolution [18,19], measurements on large epithelia [20], and determination of traction fields in 3D [21]. The basic idea is to image the displacements of the substrate caused by the cell forces and then infer the force field exerted by the cells from the known mechanical properties of the substrate. In practice, displacements are obtained by imaging fluorescent microbead markers embedded in the substrate, and then comparing the position of the markers when the substrate is deformed with reference images when the substrate is relaxed. A discrete displacement field of the substrate

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is determined using Particle Tracking Velocimetry (PTV) [14] or Particle Image Velocimetry (PIV)[20]. The equations relating forces and displacements are then inverted to determine the field of traction stresses applied by the cell on the substrate.

One of the main limitations of TFM is its spatial resolution. According to the Nyquist–Shannon sampling theorem, the resolution of the reconstructed traction field cannot exceed the resolution of the measured displacement field, which is in turn limited by the resolution of PIV or PTV from images at the given microscope resolution.

Here we present an novel approach to improve the resolution and quality of reconstructed tractions based on ideas from compressed sensing (CS). Cells attach to their surrounding matrix at localized spots (the FAs), and force is mainly applied via these adhesions. A typical size of a FA is 1 μm^2 [22], much smaller than the cell itself and than the field of view of the microscope. The typical number of FAs within one adhering cell is of the order of a few tens. Hence, the traction field exerted by a cell or tissue is approximately sparse in the spatial domain, i.e. mostly zero except at the FAs. It thus has much fewer degrees of freedom than the full dimension of the field which suggests that a smaller set of measurements should be sufficient to recover the tractions. This is exactly the idea behind CS: the number of measurements required to attain a sparse signal is roughly equal to the number of non-zero elements, rather than the total size of the signal. In particular, for sparse signals, it is possible to circumvent the Nyquist–Shannon sampling theorem. For TFM, this means that the traction field can be reconstructed at a higher resolution than the substrate displacement field. The theory of CS has demonstrated that such reconstruction can be implemented by L1-norm minimization and works even in the presence of considerable noise [23,24]. CS has been applied with great success in a wide range of fields, ranging from seismology [25] and astronomy [26] to magnetic resonance imaging [27] and biological imaging [28,29].

In the following, we apply CS to the traction reconstruction step of TFM. We show that it can both improve the quality of the reconstruction and allow faithful reconstructions at higher resolution than the displacement field. The paper is organized as follows: first, we briefly review CS and explain how it can be applied to the traction reconstruction step of TFM. Next, we compare Fourier-transform reconstructions (FT) [15] to CS reconstructions on simulated force fields with experimentally relevant traction distributions, substrate properties, and noise level, and study the quality of the CS force reconstructions as a function of noise level and undersampling factor. We also assess the importance of sparsity based on simulations with variable traction density and focal adhesion size. Finally, we apply the CS techniques to living cells with labeled focal adhesions, and demonstrate the applicability of CS undersampling to real TFM data.

1. CS applied to the traction reconstruction step in TFM

Compressed sensing deals with scenarios where the signal and measured data have a linear relationship

$$\mathbf{u} = A\mathbf{f}, \tag{1}$$

where $\mathbf{u}$ is the data and $\mathbf{f}$ the signal represented as vectors, and A is a known $m \times n$ matrix which encodes the measurement process. The goal is to recover $\mathbf{f}$. If the dimension of the data is smaller than that of the signal, i.e. $m < n$, then from basic linear algebra, Eq. (1) has no unique solution. However, if the signal is sufficiently sparse, then the solution can be *exactly* recovered by L1-norm minimization [23,24]. That is, out of the infinitely many solutions to Eq. (1) only one is sparse, and this unique solution equals the result of [23]:

$$\text{minimize } \|\mathbf{f}\|_1 \quad \text{subject to } \mathbf{u} = A\mathbf{f}. \tag{2}$$

L1-norm minimization is a convex problem and can be performed efficiently by existing algorithms. Even in the presence of noise a sparse signal may be recovered in the above manner [24]. To account for noise, Eq. (1) is replaced by $\mathbf{u} = A\mathbf{f} + \mathbf{e}$, where $\mathbf{e}$ is a noise term, and Eq. (2) becomes

$$\text{minimize } \|\mathbf{f}\|_1 \quad \text{subject to } \|\mathbf{u} - A\mathbf{f}\|_2 \leqslant \epsilon \tag{3}$$

for some sufficiently high tolerance ϵ which depends on the amount of noise (this approach is known as basis pursuit denoise). In order to maximize the quality of the CS reconstruction, ϵ should be optimized according to one or more quality measures.

To see that the reconstruction step of TFM can be cast in the form of the linear problem above, we briefly review the physics involved. For a linear medium, displacement $\mathbf{U}$ and traction force $\mathbf{F}$ vectors at positions $\mathbf{r}$, $\mathbf{r}'$ are related through

$$\mathbf{U}(\mathbf{r}) = \int G(\mathbf{r}, \mathbf{r}')\mathbf{F}(\mathbf{r}')d\mathbf{r}', \tag{4}$$

where the matrix G is a Green's function encoding the material properties of the medium. Typical experiments use substrates of polyacrylamide hydrogels or soft silicone elastomer gels, which are homogeneous, linear, and isotropic to a good approximation, such that Eq. (4) holds with $G(\mathbf{r}, \mathbf{r}') = G(\mathbf{r} - \mathbf{r}')$. If the substrate is sufficiently thick, it can additionally be treated as an infinite medium, filling the lower half-space. An analytical expression for G is then given by the Boussinesq solution [30]

$$G_{ij}(\mathbf{r}) = \frac{1+\sigma}{\pi E}\left(\frac{1-\sigma}{r}\delta_{ij} + \frac{\sigma}{r^3}r_i r_j\right), \tag{5}$$

where E and σ are the substrate Young's modulus and Poisson's ratio respectively. Although accounting for finite substrate thickness can in some cases help improve resolution for thin substrates [31], the use of the Boussinesq solution on a reasonably thick substrate (>50–100 μm) is standard. To extract traction forces from observed displacements, one needs to invert Eq. (4). Existing methods discretize the displacement and traction force fields on spatial grids. If we define indices α and β which run over the grid points as well as over the three vectorial components of the fields at each point, then Eq. (4) becomes $u_\alpha = G_{\alpha,\beta}f_\beta$, where $G_{\alpha,\beta}$ is now a number which determines the displacement u_α with location and direction given by α produced by a point force f_β with location and direction given by β. This is exactly a relation of the form of Eq. (1).

In the following, to avoid confusion, we use the term *traction forces* when talking about point forces (units of Newton), while the term *traction* with no qualification denotes the discretized traction stresses (units of Pascal).

Several methods are used for discretization and traction reconstruction. The Boundary Element Method uses an adaptive, triangular grid and performs the inversion in real space [14], while Fourier-Transform Traction Cytometry (FT) uses a regular, rectangular grid and performs numerically the inversion in Fourier space where the convolution in Eq. (4) becomes a product, facilitating much faster processing [15]. If in addition the location of the focal adhesion sites are known, e.g. through fluorescent staining, then Eq. (4) can be directly written as a sum over this finite number of sites [32,33]. Here we will focus on the case where such information is not available, and for simplicity we will consider regular, quadratic grids. However, we stress that the essential idea should be applicable to any discretization for which the traction force field can be expected to be sparse.

In all previous TFM approaches the displacement and traction grids have the same resolution. E.g. for quadratic grids, the size and shape of the displacement and traction pixel sizes are identical. Here, the CS reconstruction method allows for undersampling where the traction grid is finer than the displacement grid; traction