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An adaptive algorithm for tracking 3D bead displacements: application in biological experiments

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Abstract

This paper presents a feature-vector-based relaxation method (FVRM) to track bead displacements within a three-dimensional (3D) volume. FVRM merges the feature vector method, a technique used in tracking bead displacements in biological gels, with the relaxation method, an algorithm employed successfully in tracking bead pairs in fluids. More specifically, FVRM evaluates the probability of a bead pairing event based on the quasi-rigidity condition between the feature vectors of a bead and its candidate positions within a searching domain. Computational efficiency is improved via the introduction of an adaptive searching domain size and mismatches are reduced via a two-directional matching strategy. The algorithm is validated using simulated 3D bead displacements caused by a force dipole within a linear elastic gel. Results demonstrate a consistently high recovery ratio (above 98%) and low mismatch ratio (below 0.1%) for tracking parameter (mean bead distance/maximum bead displacement) greater than 0.73.

Keywords

three dimensional; bead tracking; two-frame; adaptive; relaxation method; feature vector

1. Introduction

Recent developments in quantitative biology, in particular, the emphasis on physical forces in biological systems, demands an ability to track particles (or cells) in three dimensional (3D) space and time (Legant et al., 2010, Hall et al., 2013, Wu et al., 2006). Examples include microrheology (Squires and Mason, 2010), where the mechanical properties of biological fluids are extracted from the motion of tracer particles embedded within it; hydrodynamics of living fluids (Koch and Subramanian, 2011, Wu et al., 2006) where many individual swimming bacteria are tracked to derive the mechanics of a living fluid – the bacterial suspension. More recently, tracking micrometer size particles in 3D has enabled a promising technology, 3D cellular traction force microscopy (3D TFM) (Legant et al., 2010, Hall et al., 2013). In 3D TFM, single animal cells are cultured within a hydrogel, and the cellular traction force is inferred from the gel deformation that results from the release of the cellular traction (typically by administering a drug that disrupts the proper function of cytoskeletal molecules). Here, micrometer size beads with one or more fluorescence channels (Hall et al., 2013, Sabass et al., 2008) are embedded within the hydrogel, and the

gel deformation is evaluated from the bead displacements. Challenges in micrometer size particle tracking in biological systems are the particle density, randomness of the particle trajectories, and constraints that limit how frequently particle positions can be recorded.

Existing 3D TFM methods determine the bead displacement field using either a Digital Volume Correlation (DVC) (Franck et al., 2007, Franck et al., 2011, Maskarinec et al., 2009, Bay, 2008) or a two frame particle tracking method (Legant et al., 2010, Legant et al., 2013, Hur et al., 2009, Delanoe-Ayari et al., 2010, Rieu and Delanoe-Ayari, 2012, Gjorevski and Nelson, 2012, Koch et al., 2012). The DVC algorithm finds the displacement of a sectioned volume by computing the cross correlation of two sub-volumes in two consecutive image frames. The spatial resolution of the displacements is determined by the size of the sub-volume, and thus is limited. In addition, the existing DVC algorithm assumes that the hydrogel deformation of the sub-volume only involves rigid spatial translation and stretch, but neglects rotation (Franck et al., 2007, Franck et al., 2011, Maskarinec et al., 2009). Assuming that there is no rotation of a gel sub-volume is a potential source of error because it is known that cells assume a complex geometry within a hydrogel, and many natively derived hydrogels have nonlinear material properties (Storm et al., 2005, Griffith and Swartz, 2006). In contrast to DVC, two frame particle tracking overcomes these limitations because a correctly matched bead pair is an accurate measure of the material displacement at a particular point.

Two frame particle tracking was originally developed for measuring fluid flows in the field of fluid mechanics (Xu, 2008, Pereira et al., 2006, Ouellette, 2006, Crocker and Grier, 1996). Micrometer size beads are seeded within a fluid flow, and displacements of bead pairs are used to compute the fluid velocity at the bead location. Pereira *et al.* recently implemented a relaxation method (Baek, 1996) tracking micrometer size beads in 3D flow fields. They found that the relaxation method compared favorably over two other commonly used tracking methods, the nearest neighbor and neural network method (Pereira et al., 2006). The relaxation method matches a bead pair by updating the probability of a matching bead pair iteratively based on a quasi-rigidity condition (i.e., neighboring beads have similar displacement vectors).

Current two frame particle tracking algorithms in the context of 3D cell traction microscopy include nearest neighbor (Koch et al., 2012, Crocker and Grier, 1996), auto-regression (Gjorevski and Nelson, 2012), and feature-vector-based (Legant et al., 2010, Legant et al., 2013) methods. Among these methods, feature-vector-based methods determine the optimal match by evaluating the similarity of the feature vectors of a bead (see section 2.2 for the definition of feature vectors) with those of its candidates. They are more straightforward to implement than auto-regression methods, and yield better matching accuracy than the nearest neighbor method especially when the bead displacement is relatively large compared to the average bead spacing. Legant *et al.* (Legant et al., 2010) first apply a feature-vector-based tracking method to 3D traction force microscopy. Because their focus is on the biological implications of traction force microscopy, details of their bead tracking method have not been published. From our private communication with Legant and Chen (Legant, 2013), we learnt that in their approach, feature vectors of a bead are formed by connecting the bead position to typically three of its nearest neighbors. A cost function is then

constructed by summing the Euclidian distances from the set of feature vectors in the reference frame to the set of feature vectors in the deformed frame. Additional filtration and smoothing steps are applied depending on the data sets to achieve high matching ratio.

In this paper, we present a 3D particle tracking method integrating the feature-vector-based method with the relaxation method. Two unique aspects of the feature-vector-based relaxation method (FVRM) are its adaptive searching domain size, which is facilitated by the use of local feature vectors; and its two-directional matching strategy. This method is validated against the analytical solution of the bead displacements caused by the release of a known force dipole within a linear elastic gel. In the supplementary material, it is compared against a modified version of original relaxation method (MR) (Pereira et al., 2006) and a modified version of original feature vector method (MFV) (Legant et al., 2010).

2. Two frame particle tracking

2.1 Problem statement and notation

In two frame particle tracking, the bead positions are known at the two time points. Our goal is to track and pair the position of each bead across time. Let Ω denote a fixed volume in space. Suppose at time t_1 , there are N_1 beads $A = \{p_1, p_2, \dots, p_{N_1}\}$ occupying Ω . At a later time $t_2 > t_1$, beads occupying the same volume Ω will be contained in the set $B = \{q_1, q_2, \dots, q_{N_2}\}$. Note that the sets A and B do not necessarily contain the same beads or the same number of beads since some beads may enter or leave volume Ω ; or some beads may not be observed in one of these configurations, e.g. some beads may be too dim to be observed at time t_2 . Such beads will be absent in B but can belong to A .

The position of a bead is measured by its coordinate vector $\vec{p}$ with respect to a Cartesian coordinate system fixed to the laboratory. We will call (p, q) a matching pair if at time t_2 , the bead p moves to the position $\vec{q}$ occupied by bead q in B . Clearly, the number of matching pairs cannot exceed $\min(N_1, N_2)$. After all the matching pairs are found, the true displacement vector of a matched bead p is given by $\vec{q} - \vec{p}$. In the following, for arbitrary bead pair (p, q) , we also call $\vec{d}_{pq} = \vec{q} - \vec{p}$ a displacement vector but it is not to be confused with the true displacement where p and q are a matching pair.

It is to be note that we have assumed no stage drift. If stage drift occurs, the observed bead positions at time t_1 and t_2 will occupy a slightly different volume in space. We will address drift correction in Section 5.

2.2 A feature-vector-based relaxation (FVRM) method

The basic assumption of FVRM is that deformation “preserves” local geometry around a bead which is represented by a set of feature vectors that connect the bead with some of its neighboring beads. Usually the more feature vectors are chosen, the more accurate but less efficient the algorithm becomes. In our implementation, a number of three feature vectors are chosen for each bead at both t_1 and t_2 . Specifically, the feature vectors of a bead $p \in A$ are determined by first finding the three nearest neighboring beads of p in A (see figure 1). Denote the set containing these three beads as V_p . The three vectors that connect $\vec{p}$ with

each of its three nearest neighbors in V_p are called the feature vectors of bead p . Note that these feature vectors are fixed at the spatial position $\vec{p}$ with respect to the laboratory frame and do not move with p . We denote $F_p \equiv \{\vec{v}_{pp'}, p' \in V_p\}$ to be the set containing these feature vectors. Likewise, for any bead q in B , let V_q be the set of its three nearest neighbors in B . The corresponding feature vectors of q are contained in $F_q \equiv \{\vec{v}_{qq'}, q' \in V_q\}$. Finally, we denote C_p to be the set containing all candidate positions of p at t_2 . Initially C_p consists of the three nearest neighbors to the spatial position $\vec{p}$ at t_2 (q_1, q_2, q_3 in figure 1). As discussed below, the set C_p can be enlarged adaptively to include more candidate positions.

The FVRM is implemented in four steps as follows:

In step 1, we initialize the probability of a bead p moving to a bead q . Specifically, let $P_k(p, q)$ denote the probability of bead p moving to a bead $q \in C_p$ at the k^{th} iteration. The probability of p not matching with any bead inside C_p is denoted by $P_k^*(p)$. By definition,

$$\sum_{q \in C_p} P_k(p, q) + P_k^*(p) = 1 \quad (1)$$

Initialization of probabilities $P_{k=0}(p, q)$ $P_{k=0}^*(p)$ follows

$$P_0(p, q) = P_0^*(p) = \frac{1}{N_p + 1}, \quad q \in C_p \quad (2)$$

where N_p is the number of beads inside C_p . Initially N_p equals three.

In step 2, we update $P_k(p, q)$ and $P_k^*(p)$ from the k^{th} iteration to the $(k+1)^{\text{th}}$ iteration. Assume $P_k(p, q)$ are known for all p and $q \in C_p$. We obtain $P_{k+1}(p, q)$ by first updating the pseudo-probabilities $\tilde{P}_{k+1}(p, q)$, which, in the original relaxation method, is given by

$$\tilde{P}_{k+1}(p, q) = P_k(p, q) \left[A + B \left(\sum_{p' \in V_p} \sum_{q' \in C_{p'}} P_k(p', q') Q(p, q, p', q') \right) \right] \quad (3)$$

where $A = 0.3$, $B = 3$ (Pereira et al., 2006) are prescribed constants and the weighting factor $Q(p, q, p', q')$ enforces the quasi-rigidity condition, i.e. beads within a small region show a similar pattern of movement (Baek, 1996). The probability of a pair is enhanced only if there are neighboring pairs satisfying the quasi-rigidity condition, which will be described below.

The original relaxation method is global since all bead pairs are linked to each other with equation (3); and therefore it is difficult to be converted into an adaptive algorithm. To formulate an adaptive method, we convert (3) into a local update formula only involving $P_k(p, q)$ as well as the feature vectors of p and q

$$\tilde{P}_{k+1}(p, q) = P_k(p, q) \left[A + B \left(\sum_{p' \in V_p} \sum_{q' \in V_q} \hat{P}_k(p', q') Q(p, q, p', q') \right) \right] \quad (4)$$

, where

$$\hat{P}_k(p', q') = P_k(p, q) \frac{1/c(\vec{v}_{pp'}, \vec{v}_{qq'})}{\sum_{q'' \in V_q} 1/c(\vec{v}_{pp'}, \vec{v}_{qq''})}, \quad (5)$$

is an approximation to the probability $P_k(p', q')$. In (5), $c(\vec{u}, \vec{v})$ evaluates the similarity of a pair of vectors $\vec{u}, \vec{v}$ and is defined by

$$c(\vec{u}, \vec{v}) = \begin{cases} \left(\frac{|\vec{u}|}{|\vec{v}|} + \frac{|\vec{v}|}{|\vec{u}|} - 2 \right) \left(\frac{1 - \cos \langle \vec{u}, \vec{v} \rangle}{\cos \langle \vec{u}, \vec{v} \rangle} \right) & \langle \vec{u}, \vec{v} \rangle < \pi/2 \\ \infty & \langle \vec{u}, \vec{v} \rangle \geq \pi/2 \end{cases} \quad (6)$$

where $\langle \vec{u}, \vec{v} \rangle$ denotes the angle between the vector pair $\vec{u}$ and $\vec{v}$. By definition, when feature vectors $\vec{v}_{pp'}$ and $\vec{v}_{qq'}$ are close in length and orientation, $c(\vec{v}_{pp'}, \vec{v}_{qq'})$ will be close to zero and therefore $P_k(p', q')$ approximates to $P_k(p, q)$. Otherwise, $P_k(p', q')$ approximates to 0.

The quasi-rigidity condition in (4) is given by

$$Q(p, q, p', q') = \begin{cases} 1 & c(\vec{v}_{pp'}, \vec{v}_{qq'}) < \omega_c \\ 0 & \text{otherwise} \end{cases} \quad (7)$$

where $\omega_c = 0.001$ is a prescribed constant.

Finally, note that the summation scope C_p in the second summation of (3) has been replaced in (4) by the fixed set V_q , which contains the neighboring beads of q . Therefore, equation (4) provides a local update of pseudo-probabilities $\tilde{P}_{k+1}(p, q)$ which depends *only* on $P_k(p, q)$ and the feature vectors of p and q . These pseudo probabilities are then normalized to yield probabilities $P_{k+1}(p, q)$, $q \in C_p$ and $P_{k+1}^*(p)$ at the $(k+1)^{th}$ iteration so that (1) is satisfied. This is accomplished by

$$\begin{aligned} P_{k+1}(p, q) &= \frac{\tilde{P}_{k+1}(p, q)}{\sum_{q' \in C_p} \tilde{P}_{k+1}(p, q') + P_k^*(p)} \\ P_{k+1}^*(p) &= \frac{P_k^*(p)}{\sum_{q' \in C_p} \tilde{P}_{k+1}(p, q') + P_k^*(p)} \end{aligned} \quad (8)$$

Assuming that these probabilities converge after J iterations, the match for bead p is selected by finding the bead q that gives the largest $P_J(p, q)$ among $q \in C_p$. If this probability is larger than a prescribed threshold w_s^h , the match is regarded to be a *highly confident* match from A to B. If the probability is smaller than w_s^h but larger than another threshold $w_s^l < w_s^h$,

the match is regarded to be a *confident match*. In the numerical implementation, $w_s^h=0.99$, $w_s^l=0.5$ are used.

As pointed out above, the scheme to update the probabilities of bead p from the k^{th} iteration to the $(k+1)^{th}$ iteration is local, and hence adaptable. For those beads with large non-matching probabilities $P_j^*(p) > 0.5$, the candidate positions searching domain C_p is expanded adaptively to include more beads. Note that equations (4) and (5) still remain the same, except that probabilities $P_{k=0}(p, q)$, $P_{k=0}^*(p)$ needs to be reinitialized using (2) and the summation in (8) will involve more terms.

In step 3, we introduce a two directional matching strategy, i.e., the procedure in step 1 and step 2 is applied to each bead q in set B to find its match in set A. This strategy is employed to handle the situation when different beads at t_1 are matched to the same bead at t_2 .

In step 4, we describe the criterion to finally accept a pair. Specifically, for a pair found in both directions, it is accepted as a matching pair if both of following conditions are satisfied

1. the pair is a highly confident match from at least one direction or the pair is a confident match from both directions;
2. the distance between this pair of beads is less than the prescribed maximum displacement d_m .

In the original relaxation method (Baek, 1996, Pereira et al., 2006), the above two-directional matching strategy is not employed. Therefore different beads at t_1 could be matched to the same bead at t_2 , which causes inconsistent bead displacements and difficulty in calculating both recovery and mismatch ratios (see next section for definition). To provide a fair comparison between the relaxation method and FVRM, we incorporate the original relaxation method with the two-directional matching and call it the modified relaxation (MR) method. In the supplementary material, we present a comparison of FVRM with MR using simulated data from two cases. One is the Burger's vortex flow case studied previously by Pereira *et al.* (Pereira et al., 2006) and the other is the dipole forces case described in Section 4.

3. Tracking parameters

The performance of a tracking scheme deteriorates when the maximum bead displacement $|\mathbf{u}_{max}|$ between time steps becomes comparable to the mean bead distance. To quantify this dependence, we adopt the tracking parameter proposed in (Pereira et al., 2006)

$$\Phi = d_0 / |\mathbf{u}_{max}| \quad (9)$$

where d_0 is the mean bead distance in the observation volume Ω , i.e.,

$$d_0 = \left(\frac{3\Omega}{4\pi \min(N_1, N_2)} \right)^{1/3} \quad (10)$$

Generally, the performance of a tracking scheme improves as the tracking parameter increases.

To evaluate the performance of our tracking scheme, we introduce two dimensionless parameters, namely: the recovery ratio η_r and the mismatch ratio η_m . Denote $N_{cp} = \min(N_1, N_2)$ to be the actual number of bead pairs in the volume. Suppose a total number of M_p $\min(N_1, N_2)$ pairs are identified by an algorithm, amongst which M_{cp} are correct matches while the rest $M_{sp} = M_p - M_{cp}$ are incorrectly matched (spurious pairs). The mismatch ratio η_m is defined as the number of spurious pairs M_{sp} divided by the total number of pairs found M_p , i.e.,

$$\eta_m = \frac{M_{sp}}{M_p} \quad (11)$$

The recovery ratio η_r is defined as the number of correct pairs M_{cp} divided by the actual number of bead pairs N_{cp} , i.e.,

$$\eta_r = \frac{M_{cp}}{N_{cp}} \quad (12)$$

As pointed out by Pereira *et al.* (Pereira et al., 2006), η_r directly measures the matching ability of a tracking scheme and η_m indicates how well a tracking scheme discriminates among candidates and dismisses unpaired beads.

4. Numerical experiments and discussion

4.1. Tracking displacement field due to a force dipole

In cell traction experiment, certain cell types will polarize in space assuming an elongated shape. Forces exerted by such polarized cells are primarily exerted in the direction of elongation and can be approximated by equal and opposite forces at the two cell tips. Motivated by this observation, we test the FVRM by simulating the displacement field induced by a force dipole in a linearly elastic gel. As long as the cell is small in comparison with its surroundings, the induced displacement field can be modeled using the exact solution of a force dipole in an infinite elastic solid. Let x_i , $i = 1, 2, 3$ be the Cartesian coordinates of a material point in the gel. The displacement field u_i due to a force dipole of magnitude P located at $(0, 0, \pm a)$ is given by (Love, 1944)

$$\begin{aligned} \bar{u}_i &= \bar{C} \left(\frac{\bar{x}_i (\bar{x}_3 + 1)}{\bar{r}_1^3} - \frac{\bar{x}_i (\bar{x}_3 - 1)}{\bar{r}_2^3} \right), i=1, 2 \\ \bar{u}_3 &= \bar{C} \left[\left(\frac{3-4\nu}{\bar{r}_1} + \frac{(\bar{x}_3+1)^2}{\bar{r}_1^3} \right) - \left(\frac{3-4\nu}{\bar{r}_2} + \frac{(\bar{x}_3-1)^2}{\bar{r}_2^3} \right) \right] \\ \bar{C} &= \frac{(1+\nu)\bar{P}}{8\pi(1-\nu)}, \bar{r}_1 = \sqrt{\bar{x}_1^2 + \bar{x}_2^2 + (\bar{x}_3+1)^2}, \bar{r}_2 = \sqrt{\bar{x}_1^2 + \bar{x}_2^2 + (\bar{x}_3-1)^2} \end{aligned} \quad (13)$$

where we have normalized all variables using

$$\bar{x}_i = \frac{x_i}{a}, \bar{u}_i = \frac{u_i}{a}, \bar{P} = \frac{P}{Ea^2} \quad (14)$$

to reduce the number of parameters in the simulation to two, i.e., $\bar{P}$ and ν . In equations (13) and (14), E is the Young's modulus of the gel and ν is the Poisson's ratio. In the numerical experiment, these normalized parameters are chosen to be $\bar{P} = 1.5$, $\nu = 0.2$. Note that the displacement field is unbounded at the two dipole points ($r_1 = 0$, $r_2 = 0$).

Simulations are carried out with N beads uniformly sampled in a normalized cubic domain $[-2, 2] \times [-2, 2] \times [-2, 2]$ excluding the region where the magnitude of the normalized displacement vector exceeds 0.2, i.e., the maximum bead displacement $|\mathbf{u}_{\max}| \approx 0.2$. Since bead displacements given by (13) become unbounded at $(0, 0, \pm 1)$, the tracking performance at locations close to $(0, 0, \pm 1)$ is expected to be worse. We categorize all beads into two categories: I) beads with small displacements $|\mathbf{u}| \leq 0.3 |\mathbf{u}_{\max}|$ where $|\mathbf{u}|$ is the magnitude of the displacement vector of a particular bead; II) beads with large displacements $|\mathbf{u}| > 0.3 |\mathbf{u}_{\max}|$. Figure 2 shows the recovery and mismatch ratios of the algorithm for beads in categories I and II respectively as well as the total population. Figure 3 shows the computation time of the algorithm. In both figure 2 and 3, each point represents a particular bead number N from 1000, 2000, 3000, 4000, 5000, 10000, 20000, 40000. For each N , three different realizations of bead positions are sampled in the volume, which are used to obtain the error bars in the figures. For each realization, the tracking parameter is calculated using in which the mean bead distance d_0 is found by directly averaging the distances between a bead and its nearest neighbor. The average tracking parameters of the three realizations are used in the figures to characterize the different bead densities. For $N = 5000$, the average tracking parameter is 0.73 (the fourth data point from left to right). In real experiments, this corresponds to a bead density of 5.0×10^9 beads / ml. Here the maximum bead displacement is assumed to be $5\mu\text{m}$ and the mean bead distance is estimated using (10). As shown in figure 2, for tracking parameter greater than 0.73, the overall recovery ratio is above 98% and the mismatch ratio is below 0.1%. It takes less than 2 minutes for our Matlab (The MathWorks Inc., Natick, MA) code to finish tracking for $N = 5000$, as shown in figure 3. Here all computations are carried out on a desktop with an AMD FX(tm)-8150 Eight-core Processor (3.6GHz) and 32GB RAM. As we increase the bead density, the tracking parameter falls below 0.73, causing deterioration in both recovery and mismatch ratios and increase in computation time. This is mainly due to frequent expansion of candidate searching domain and poorer tracking performance of beads with displacements large compared with the bead spacing.

4.2. Effect of observation errors

In experiments, bead positions are subjected to observation errors, e.g., optical/electronic noise and image reconstruction error. We investigate this effect on the performance of our tracking scheme by introducing a random perturbation in the bead positions at both time t_1 and t_2 . We use the dipole forces case with $N = 5000$ as an error-free reference. The tracking parameter for this system is 0.73. We apply perturbation on each bead using a uniform

distribution in the interval $[-\frac{1}{2}\delta d_0, \frac{1}{2}\delta d_0]$ in the x-y directions, and $[-\frac{3}{2}\delta d_0, \frac{3}{2}\delta d_0]$ in the z direction, where d_0 is the mean bead distance and δ is a dimensionless parameter which can be interpreted as a scaled noise amplitude. Figure 4 shows that the performance of the tracking scheme gradually deteriorates as increasing noise undermines the validity of quasi-

rigidity condition. Therefore, it is crucial to obtain accurate 3D bead positions from raw images in order to achieve reliable matching performance. This is facilitated by the sub-pixel localization technique introduced by Gao *et al.* (Gao Y, 2009). With the technique, the noise amplitude due to image reconstruction is less than $0.05\ \mu\text{m}$ for a pixel size of $0.2\ \mu\text{m}$. For mean bead distance $d_0 = 2.5\ \mu\text{m}$, the corresponding scaled noise amplitude is less than 4%. For these cases, figure 4 shows that the recovery ratio is above 95% and the mismatch ratio is below 0.1% using FVRM.

5. Drift correction

In our matching algorithm we have assumed that there is no drift in the frame of reference from one time-point to the next. This assumption allows us to use a fixed coordinate system to specify the position of a bead. In real microscopy experiments, temperature changes in the imaging system and stage actuation error can result in drift of the optical focal plane and therefore the frame of reference between time-points.

Suppose $A = \{p_1, p_2, \dots, p_{N_1}\}$ and $B = \{q_1, q_2, \dots, q_{N_2}\}$ correspond to the two sets of beads observed in volume Ω at time t_1 and t_2 respectively. The measured positions of beads in sets

A and B are $\{\vec{p}_i\}$ and $\{\vec{q}_i + \vec{d}\}$, where $\vec{p}_i, \vec{q}_i$ are bead positions with respect to a same fixed laboratory coordinate system and $\vec{d}$ is the drift at time t_2 . If the drift is large in comparison with mean bead distance, the above tracking scheme will break down. Therefore, it is necessary to correct the drift before feeding bead positions into the tracking algorithm.

The first step of drift correction is to provide an initial estimate of drift. In cell experiments, most of the gel volume experiences very little displacement. The initial estimate of drift can therefore be obtained by recording bead positions across time in those regions.

Alternatively, the position of a feature fixed to the laboratory reference frame, such as a fluorescent bead attached to the cover glass, can be imaged at each time-point to calculate the drift. Once an initial drift $\vec{d}_0$ is obtained, the drift is subtracted from all bead positions and the above tracking algorithm is applied to drift-corrected bead positions $\{\vec{p}_i\}$ and $\{\vec{q}_i + \vec{d} - \vec{d}_0\}$. As long as the initial drift estimate is sufficiently accurate, the matching algorithm should be able to match most bead pairs correctly. A correction of the drift estimate can then be obtained by averaging the matched bead displacements in the far field where displacements are expected to be small. This procedure can be performed iteratively until the drift correction is sufficiently refined.

6. Concluding remarks

To conclude, we introduce FVRM as an efficient and accurate tracking algorithm especially suitable for biological systems where displacements are non-uniformly distributed. It extends the feature-vector-based algorithm first proposed by Legant *et al.* (Legant et al., 2010) by accounting for angle differences in the cost function and integrates with the relaxation method (Baek, 1996, Pereira et al., 2006) that is previously used in the fluid mechanics community. FVRM adaptively expands the size of its candidate positions

searching domain and is therefore efficient especially for cell (Legant et al., 2010, Hall et al., 2013) and indentation experiments (Hall et al., 2012). In these experiments, bead displacements are non-uniformly distributed and most of the region experiences small displacements.

We compare FVRM with a modified version of original relaxation method (MR) (Pereira et al., 2006) and a modified version of original feature vector method (MFV) (Legant et al., 2010) using simulated displacement field induced by a force dipole (see supplementary material). The results show that FVRM and MR perform well for large tracking parameters (> 0.73) with a high recovery ratio (above 98%) and low mismatch ratio (below 0.1%). For a small tracking parameter (< 0.73), MR performs slightly better in terms of recovery and mismatch ratios but its time cost increases dramatically. For example, at tracking parameter of 0.5, FVRM is about 30 times faster than MR. Although MFV overall does not perform as well as FVRM or MR both in terms of recovery and mismatch ratios, we note here that MFV is a re-implementation of the original feature vector method proposed by Legant *et al.*, and their detailed postprocessing procedure is not included in our computation. It is also to be mentioned that both FVRM and MFV can be improved by using more than three feature vectors, but at the cost of greater computation time. In real experiments, the performance of tracking algorithms is undermined by observation errors. As shown in the numerical experiment of dipole forces case, for tracking parameter greater than 0.73, the effect of observation error using the sub-pixel localization technique (Gao Y, 2009) on the tracking performance of FVRM is minor as long as the mean bead distance is greater than 2.5 μm .

Finally, we point out that above tracking algorithms can be naturally used to track beads with two or more fluorescence channels (Sabass et al., 2008). By tracking the beads in different channels separately, each channel has a lower bead density, or equivalently, larger tracking parameter which will lead to lower computation time and better matching efficiency. Therefore a denser and more precise sampling of displacement field could be achieved compared to the traditional single channel approach.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- BAEK SJ, L SJ. A new two-frame particle tracking algorithm using match probability. *Experiments in Fluids* 1996; 22:23–32.
- BAY BK. Methods and applications of digital volume correlation. *The Journal of Strain Analysis for Engineering Design*. 2008; 43:745–760.

- CROCKER JC, GRIER DG. Methods of digital video microscopy for colloidal studies. *Journal of colloid and interface science*. 1996; 179:298–310.
- DELANOE-AYARI H, RIEU JP, SANO M. 4D Traction Force Microscopy Reveals Asymmetric Cortical Forces in Migrating Dictyostelium Cells. *Physical Review Letters*. 2010; 105
- FRANCK C, HONG S, MASKARINEC S, TIRRELL D, RAVICHANDRAN G. Three-dimensional Full-field Measurements of Large Deformations in Soft Materials Using Confocal Microscopy and Digital Volume Correlation. *Experimental Mechanics*. 2007; 47:427–438.
- FRANCK C, MASKARINEC SA, TIRRELL DA, RAVICHANDRAN G. Three-dimensional traction force microscopy: a new tool for quantifying cell-matrix interactions. *PloS one*. 2011; 6
- GAO Y, K ML. Accurate detection and complete tracking of large populations of features in three dimensions. *Optics express*. 2009; 17:4685–704. [PubMed: 19293898]
- GJOREVSKI N, NELSON CM. Mapping of Mechanical Strains and Stresses around Quiescent Engineered Three-Dimensional Epithelial Tissues. *Biophysical Journal*. 2012; 103:152–162. [PubMed: 22828342]
- GRIFFITH LG, SWARTZ MA. Capturing complex 3D tissue physiology in vitro. *Nature reviews Molecular cell biology*. 2006; 7:211–24.
- HALL MS, LONG R, FENG X, HUANG Y, HUI C-Y, WU M. Single Cell Traction Microscopy within 3D Extracellular Matrices. *Experimental Cell Research*. 2013 Submitted.
- HALL MATTHEWS, LONG R, HUI C-Y, WU M. Mapping Three-Dimensional Stress and Strain Fields within a Soft Hydrogel Using a Fluorescence Microscope. *Biophysical Journal*. 2012; 102:2241–2250. [PubMed: 22677377]
- HUR SS, ZHAO Y, LI Y-S, BOTVINICK E, CHIEN S. Live Cells Exert 3-Dimensional Traction Forces on Their Substrata. *Cellular and Molecular Bioengineering*. 2009; 2:425–436. [PubMed: 19779633]
- KOCH, DL.; SUBRAMANIAN, G. Collective Hydrodynamics of Swimming Microorganisms: Living Fluids. In: DAVIS, SH.; MOIN, P., editors. *Annual Review of Fluid Mechanics*. Vol. 43. 2011.
- KOCH TM, MUNSTER S, BONAKDAR N, BUTLER JP, FABRY B. 3D Traction Forces in Cancer Cell Invasion. *Plos One*. 2012; 7
- LEGANT WR, CHEN CS. Private Communication. 2013
- LEGANT WR, CHOI CK, MILLER JS, SHAO L, GAO L, BETZIG E, CHEN CS. Multidimensional traction force microscopy reveals out-of-plane rotational moments about focal adhesions. *Proceedings of the National Academy of Sciences of the United States of America*. 2013; 110:881–6. [PubMed: 23277584]
- LEGANT WR, MILLER JS, BLAKELY BL, COHEN DM, GENIN GM, CHEN CS. Measurement of mechanical tractions exerted by cells in three-dimensional matrices. *Nature methods*. 2010; 7:969–71. [PubMed: 21076420]
- LOVE, AEH. A treatise on the mathematical theory of elasticity. New York: Dover Publications; 1944.
- MASKARINEC SA, FRANCK C, TIRRELL DA, RAVICHANDRAN G. Quantifying cellular traction forces in three dimensions. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 104:22108. [PubMed: 20018765]
- OUELLETTE N, X H, B E. A quantitative study of three-dimensional Lagrangian particle tracking algorithms. *Experiments in Fluids*. 2006; 40:301–313.
- PEREIRA F, STÜER H, GRAFF EC, GHARIB M. Two-frame 3D particle tracking. *Meas Sci Technol Measurement Science and Technology*. 2006; 17:1680–1692.
- RIEU JP, DELANOE-AYARI H. Shell tension forces propel Dictyostelium slugs forward. *Physical Biology*. 2012; 9
- SABASS B, GARDEL ML, WATERMAN CM, SCHWARZ US. High Resolution Traction Force Microscopy Based on Experimental and Computational Advances. *Biophysical Journal*. 2008; 94:207–220. [PubMed: 17827246]
- SQUIRES TM, MASON TG. Fluid Mechanics of Microrheology. *Annual Review of Fluid Mechanics*. 2010

- STORM C, PASTORE JJ, MACKINTOSH FC, LUBENSKY TC, JANMEY PA. Nonlinear elasticity in biological gels. *Nature*. 2005; 435:191–194. [PubMed: 15889088]
- WU MM, ROBERTS JW, KIM S, KOCH DL, DELISA MP. Collective bacterial dynamics revealed using a three-dimensional population-scale defocused particle tracking technique. *Applied and Environmental Microbiology*. 2006; 72:4987–4994. [PubMed: 16820497]
- XU H. Tracking Lagrangian trajectories in position-velocity space. *Measurement Science & Technology*. 2008; 19

Figure 1.

Overlapped bead positions at t_1 and t_2 . p is a bead at time t_1 occupying the spatial position $\vec{p}$. Feature vectors $\vec{v}_{pp_1}$, $\vec{v}_{pp_2}$, $\vec{v}_{pp_3}$ connect p with its three nearest neighbors at time t_1 . The candidate positions searching domain C_p includes the three nearest beads q_1 , q_2 , q_3 at time t_2 to the spatial position $\vec{p}$. Dotted arrow lines indicate the feature vectors of one of the candidates q_3 .

Figure 2.

Dipole forces case. Plots of recovery ratio (left) and mismatch ratio (right) versus the tracking parameter for small displacement beads (category I), large displacement beads (category II) and overall population (I+II). Points from right to left represent respectively $N = 1000, 2000, 3000, 4000, 5000, 10000, 20000$ and 40000 beads sampled in the cube. For each N , error bars are obtained from three different realizations of bead positions.

Figure 3.

Plot of the computation time of FVRM for the dipole forces case versus the tracking parameter. The Matlab code is run on a desktop with an AMD FX(tm)-8150 Eight-core Processor (3.6GHz) and 32GB RAM.

Figure 4.

Effect of random perturbation of bead positions on the recovery ratio η_r and mismatch ratio η_m . The tracking parameter for the error free reference system is 0.73. For each scaled noise

amplitude δ , noise in the x-y directions is uniformly distributed in $[-\frac{1}{2}\delta d_0, \frac{1}{2}\delta d_0]$. In the z direction, the noise amplitude is three times larger. Error bars are obtained from three independent realizations of perturbed bead positions.