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Comparison of Intensity Based Similarity Measures for Matching Genomic Structures in Microscopic Images of Living Cells

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***Abstract*—This paper presents our comparative study of the application of intensity based similarity measures to the problem of matching genomic structures in microscopic images of living cells. As part of our ongoing research [7], [8] we present here for the first time evidence from experiments and simulations that show the benefit of using an iterative matching algorithm guided by an intensity based similarity measure. Our experimental results are compared against a gold standard and suggest the measures that work best in the presence of fluorescent decay and other problems inherent to time-lapse microscopy. This makes our approach widely applicable in the study of the dynamics of living cells with time-lapse microscopic imaging.**

I. Introduction

RECENT progress in technology, specifically in the areas of fluorescent labeling, microscopy and image analysis [1]–[4] has enabled the detailed study of vital processes within living cells leading to breakthroughs in our understanding of fundamental processes such as DNA replication, and the organization of chromatin and its relation to the functions of living cells. In this research effort quantitative data are produced from measurements of temporal changes in size, location and brightness of objects imaged by the microscope. Our newly developed computational approach, initially described in [7] and [8] has been applied to the matching of genomic structures between sequential time-point images for registration and measurement of changes in living cells over time.

The purpose of image registration is to establish spatial correspondence. The problem of registration can be separated into two distinct but complementary tasks: (1) matching or forming correspondences between the two images to be registered and (2) computing transformation that aligns the two images according to an optimizing criterion. The Iterative Closest Point (ICP) algorithm [5], [6] combined these two tasks so that they could be done in tandem and iteratively until some convergence criterion (usually the estimate of registration error below a certain tolerance) is met. To state this formally, if I_1 and I_2 are the two images to be registered and if T is the transformation mapping the coordinate system of I_1 onto the coordinate system of I_2 then ICP iterates on two main steps: (1) using a fixed estimate, T the transformation is applied to each point (typically control points) from the image I_1 and the closest point (according to some criterion which is usually the Euclidean distance) in image I_2 is detected as a temporary match, and (2) using constraints formed from these matches, a new best estimate T is computed. This process is repeated until the estimate T stabilizes or registration error falls below a tolerance. We have developed an image registration approach [7], [8] based on the framework of ICP and made robust by the incorporation of intensity based similarity measures. The algorithm has been tested in living cell studies where each image of a time-lapse microscopic image sequence is first segmented by algorithms described earlier [1], [2], [8] and matched in chain by our approach to extract scientific information regarding living cell dynamics and chromatin movement [7]. In this paper we present for the first time a comparative analysis of some important similarity measures used in our approach.

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II. Iterative Image Matching and Registration

A. The Image Registration Problem

Digital image matching establishes the correspondences between primitives extracted from two or more digital images depicting at least partly the same scene. The primitives can be gray level windows or features extracted from the images. The problems inherent to image matching are: (1) ambiguity of solutions if local information is used and (2) high computational costs. Cross correlation is a widely used measure in which a template window is matched against several other possibly corresponding windows by calculating the cross correlation coefficient ρ between the

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template window and the corresponding window. The best matched window gives rise to the maximum ρ. Other popular cost functions such as sum of absolute differences and sum of squared differences have been used in the literature [9]–[12]. However the restrictive assumption behind such functions is a linear relationship between intensity values in the two images. This is in general not valid in our case since fluorescent decay can lead to intensity changes that do not follow a linear model.

The image matching problem is inherently an ill-posed problem due to various reasons that are application domain specific. In some cases a correspondence may not exist due to occlusion or the severity of noise may introduce incorrect correspondences. In our case the problem is ill-posed mainly because: (1) a genomic structure present in one time-point may disappear (exit) in the next time-point or a structure that was not present may appear (enter) at the next time-point and (2) the images we wish to consider have a high density of structures within the field of view. Problem (1) occurs for example in PCNA-GFP sites [13] where the structures dynamically appear and disappear, although biological estimates of the percentage (typically very low) of such dynamic sites amongst a given population can be made. The fact that (2) is true in most cases can be understood from the fact that genomic material (DNA, proteins etc.) are tightly packed inside the cell nucleus and the chemical staining process is highly successful in binding fluorescent dyes to almost all such genomic molecules. Fortunately algorithms can be proposed for ill-posed problems by introducing domain specific knowledge about the problem. In our case we can state the following:

1. the intensities of images for a single sequence have been acquired using the same spectral band
2. the genomic structure undergo fluorescent decay, i.e. overall their individual gray values diminish in value and this can be partially corrected for with standard software provided with microscope apparatus
3. heterogeneity and individuality of intensity distributions, when comparing one structure to another in a small neighborhood, is maintained over time

When designing an image matching algorithm the following important questions have to be answered:

1. which primitives to use for matching?
2. what geometric transformation or intensity mapping function is most suitable?
3. how is the similarity between primitives measured?
4. how is the optimal match computed?

Our approach to match is based on a combination of *feature based matching* and *area based matching* in which we use structure centers as control points and use intensity values within a structure (as outlined by segmentation) to perform intensity based matching of point distributions.

B. Calculation of the Intensity Based Match

Several methods have been explored in literature for calculating the degree of intensity based match between entire images or small portions of them. They either directly compare the intensities [9]–[12] voxel by voxel, sometimes for multimodality images [14] by finding correspondences in intensity ranges between different modalities, or use the principles of information theory [15], [16] to minimize the joint entropy [17], [18]. However the most important breakthrough from the information theoretic approaches came when the concept of maximization of mutual information was applied [19]–[23] to overcome certain shortcomings of the joint entropy approach. More recent and specific problem domain oriented work includes [24]–[27] which show the ill-posed nature of the problem and the constraints that have to be taken into account to devise solutions. Our approach here is to use intensity similarity within the iterative framework for registering point sets. For this purpose we will list some of the effective ones, outline their limitations and propose the measure that we have found to be most suited to the problem presented here. We present experimental results on images and compare them with a gold standard, namely manual matching by an expert. Results of simulation are also shown to back our arguments.

III. MEASURES OF SIMILARITY

A. Histogram Based Measures of Similarity

In this section we will look at several methods to measure the similarity between histograms $H = \{h_i\}$ and $K = \{k_i\}$. The Minkowski distance [28]–[30], being the L_n distance between the two histograms is probably the simplest:

$$d_{L_n}(H,K) = \left(\sum_i |h_i - k_i|^n \right)^{1/n}.$$

The χ^2 statistics [28]–[30] measures how unlikely it is that one distribution was drawn from the population represented by the other. It is given by

$$d_{\chi^2}(H,K) = \sum_i \frac{(h_i - m_i)^2}{m_i}, \text{ where } m_i = \frac{(h_i + k_i)}{2}.$$

Use of these bin-by-bin measures is not useful for small images or portions of images where perceptually similar images could have histograms that are slightly different and could still give rise to large differences measured by the previous equations. This happens because neighboring bins are not compared across histograms. For example in worst cases the Minkowski distance will evaluate to 2 and the χ^2 distance to 1 even when the images are perceptually similar.

To overcome the shortcomings of bin-by-bin similarity measures, the L_1 distance between cumulative histograms

also known as the match distance was used [28]–[30]:

$$d_{CDF}(H,K)=\sum_i\left|\hat{h}_i-\hat{k}_i\right|,$$

where $\hat{h}_i=\sum_{j\le i}h_j$ is the cumulative histogram of $\{h_i\}$ and similarly for $\{k_i\}$. The Kolmogorov-Smirnov distance, instead of summing over differences is defined as the maximum difference between the two cumulative histograms [28]–[30]:

$$d_{KS}(H,K)=\max_i\left(\left|\hat{h}_i-\hat{k}_i\right|\right)$$

B. *Information Theoretic Measure of Similarity*

The relative entropy or the Kullback-Leibler divergence is defined as:

$$D(p\parallel q)=\sum_{x\in X}p(x)\log_2\frac{p(x)}{q(x)}$$

$p(x)$ and $q(x)$ are two probability mass functions over the random variable X. The KL divergence is a measure of the distance between the two distributions or equivalently, it is the inefficiency of assuming that the distribution of X is $q(x)$ when the true distribution is $p(x)$ [15]. The KL divergence is non-negative. It is zero if and only if $q(x)$ and $p(x)$ are identical distributions.

We apply the Kullback-Leibler divergence in the following way: estimate the probability distribution function of the gray values within an object contour by maximum likelihood estimation [28]–[31] and then use the estimated parameters of the distributions to calculate the KL divergence in a single step. To elaborate, the Gamma probability distribution function (p.d.f) is used for this purpose. The justification for using the Gamma p.d.f is that it is one of the most flexible distribution functions that can fit statistical data [31]–[34]. We also performed goodness of fit tests on randomly chosen structures and found that the Gamma distribution fitted the data better than other standard distributions like the normal p.d.f. The shape and location of the Gamma p.d.f are a governed by two parameters *b* and *c* which contribute to the flexibility of the distribution [28]–[34]:

$$y(x)=G(x;b,c)=\frac{1}{\Gamma(c)}\frac{x^{c-1}}{b^c}e^{-\frac{x}{b}}.$$

The KL divergence between two such Gamma p.d.f's, $q(x)=G(x;b_q,c_q)$ and $p(x)=G(x;b_p,c_p)$ is given in closed form by:

$$KL_G(b_q,c_q;b_p,c_p)=(c_q-1)\Psi(c_q)-\log b_q-c_q-\log\Gamma(c_q)+\log\Gamma(c_p)+c_p\log b_p-(c_p-1)(\Psi(c_q)+\log b_q)+\frac{b_qc_q}{b_p}$$

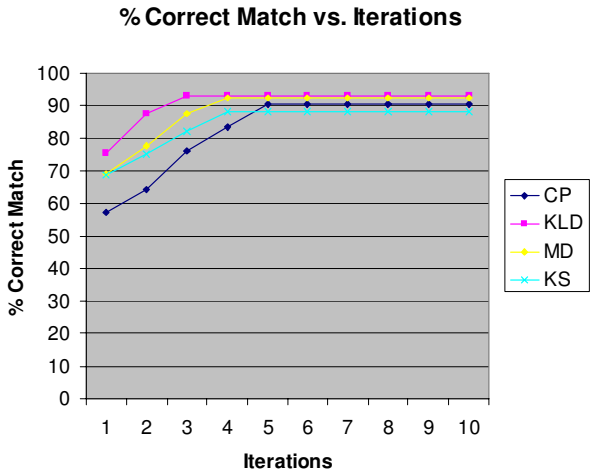

Fig. 1. Percent correct matches versus number of iterations of the iterative algorithm with closest points and intensity similarity measures.
Series details: CP = Closest Points, KLD = Kullback-Leibler Divergence, MD = Match Distance, KS = Kolmogorov Smirnov Distance

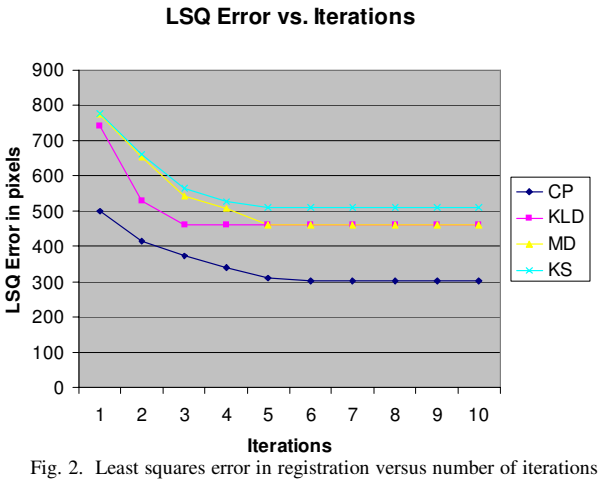

Fig. 2. Least squares error in registration versus number of iterations of the iterative algorithm with closest points and intensity similarity measures.
Series details: CP = Closest Points, KLD = Kullback-Leibler Divergence, MD = Match Distance, KS = Kolmogorov Smirnov Distance

IV. EXPERIMENTAL RESULTS

We show representative results [Fig. 1] and [Fig. 2] of applying our algorithm to time-lapse image sequences of living cells such as [Fig. 4]. Two image sequences, each containing thirty time-point images, were tested. A total of ten adjacent time-point images showing approximately three hundred sites at each time-point were taken and manually matched in pairs by a human expert. The correspondences were recorded for measuring the correctness of the matches computed by different implementations of the matching algorithm. We compare matches based on the Closest Point (CP), Kullback-Leibler divergence (KLD), Match Distance

(MD) and the Kolmogorov-Smirnov (KS) measure. Each graph shows the change in percent correct matches against the number of iterations. Also shown are the least square (LSQ) errors at each stage of iteration for all the match heuristics.

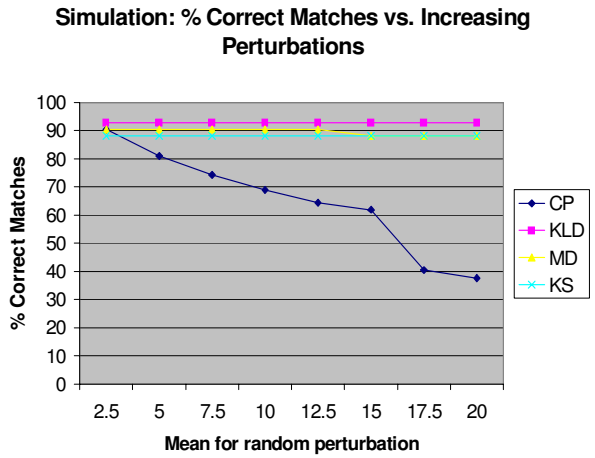

Fig. 3. Simulation of structural mismatch by perturbing chromatin domains by randomly selecting distances from a normal distribution. Series details: CP = Closest Points, KLD = Kullback-Leibler Divergence, MD = Match Distance, KS = Kolmogorov Smirnov Distance

V. SIMULATIONS

We present simulations [Fig. 3] to test the robustness of the algorithm with increasing structural mismatch. For this purpose we took a pair of control point sets and their structure intensities from a pair of adjacent time-point images and perturbed the positions of the control points by distances picked randomly from a normal distribution. The percent correct matches are plotted against increasing standard deviation for the normal distribution. Note that the radius of neighborhood used for searching in the intensity match heuristics were changed to match the increasing perturbations.

VI. CONCLUSION AND FUTURE WORK

We have demonstrated that adding an intensity similarity adds robustness to the iterative scheme for registration. While we have shown results on representative time sequences, its effectiveness is shown in the simulation results which clearly show a marked decrease in the performance of the closest point heuristic compared to the intensity match heuristic with increasing structural dissimilarity. Also it is worth noting that while the point based heuristic gets stuck in wrong matches that have lower least squares error than the correctly formed matches of the intensity heuristic. It is also worthwhile to compare our approach to other information theoretic approaches, specifically mutual information based approaches [35]. Here registration is assumed to correspond to maximization of mutual information: the images have to be aligned in such a manner that the amount of information they contain about each other is maximal. In this sense the Kullback Leibler divergence is used to measure the distance between the joint distribution of the images' intensities and the joint distribution in the case of independent images. In other words the divergence decreases as there is more mis-registration. In contrast our approach seeks to match images based on the intensity information content in specific spatial locations within the images. The KL divergence is used as a means to measure the degree of similarity in the intensity distributions at those locations. A future extension of this work could involve extending our approach by matching larger cell structures (typically larger areas such as the nuclear periphery) with suitable heuristics in order to initialize the iterative matching process.

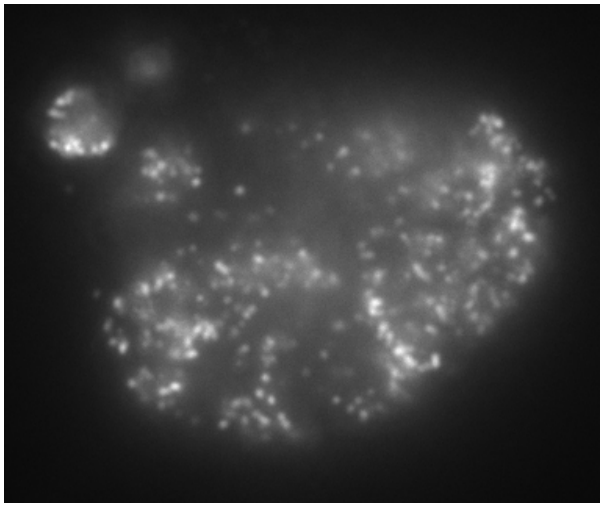

Fig. 4. A living cell image showing chromatin domains at a particular time-point in the early synthetic phase of the cell cycle.

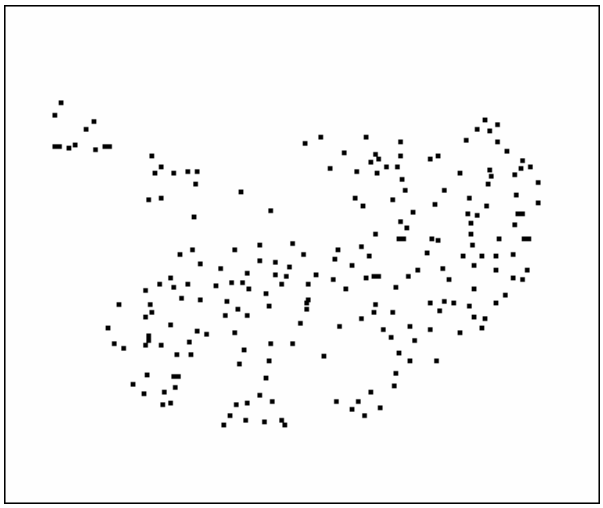

Fig. 5. Control points of the image in Fig. 4 obtained from segmentation.

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