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Automatic detection of colloidal particles based on a shape regularized integrated active contour model

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ABSTRACT

Manual segmentation of single colloidal particle in suspension encounters a bottleneck when a number of defocused particles simultaneously exist in an image. In this paper, we describe an image processing algorithm for extracting individual particle from digitized microscope images of colloidal suspensions. We propose a particle detection and location solution using a shape regularized integrated active contour model (ACM). Compared with existing methods where active contour models are not applied well to deal with multiple objects in complicated background, the proposed approach can automatically identify and locate multiple particles by combining characteristics of the particles such as shape, boundary and region. A regularization term is defined by prior information of specific shape, which is able to drive the shape of evolving curve toward the shape prior gradually. To locate the centers of the particles, the Hough transform is applied. Experimental results using polystyrene beads as sample particles reveal that the method has high efficiency and ability to deal with colloidal particles.

1. Introduction

The analysis of microparticles' positions has great significance in a broad range of research fields. Indeed, particle-tracking microscopy [1] enables researchers to study the dynamics of diverse small objects ranging from microscale colloids to nanoscale molecular motor proteins [2,3]. To understand colloidal particles' dynamical behaviors in suspensions, we need to measure the interaction between them by particle tracking with manipulating techniques. This will have an immediate impact on the fabrication of nanostructured materials. In biotechnology and modern cell biology, accurate location of fluorescent particles contributes to the observation of intra cellular transport processes, e.g. the diffusion and interaction of vesicles. In the study of fluid flow, the particle location tracking can effectively give three components three-dimensional (3C3D) images [4] of fluid movement.

Micron-size particles in suspensions undergoing Brownian motions [5] can be imaged through a light microscope. In some experiments, the diffusive motion of an isolated colloidal sphere has been studied by imaging and tracking the sphere's motion using digital video microscopy [6]. However, when a large number of particles concurrently spread in suspension, the acquired images may suffer from cluttered background and some particles are out of focus. Fig. 1 shows an image with some defocused particles. This brings difficulty to analyze the particles' position and motion.

Extracting individual particle is the first step in image analysis. A number of methods for automatic particle detection have been proposed in recent years, e.g., template matching (a matched filter) [7], local comparison of pixel intensity values [8], quantitative measures of the local image statistics [9], and a three-layer neural network algorithm [10]. The technique of edge detection is an independent method for particle identification, but its application to particle images has not been fully explored [11].

Although edge detection in particle images remains problematic, we find that active contour models (ACMs) have the ability to utilize prior knowledge of particle shapes and exert much stronger global constraints. This characteristic can be used successfully in particle images. ACMs for segmentation aim to drive the curves to reach the boundaries of the interested objects. The driven forces are mainly from the image data, including edge-based [12,13] and region-based forces [14,15]. Most ACM methods are not intrinsically able to deal with occlusion problems or presence of cluttered background, therefore, it is needed to incorporate prior shape information into active contour models. However, a limitation of shape prior segmentation methods [16,17] is that they cannot be applied to the simultaneous segmentation of multiple independent objects. Moreover, they easily suffer from serious boundary leakage for images with weak boundaries. Additionally, the nonlinear convergence of the evolving curve increases the computational complexity. In this paper, our goal is to develop an effective

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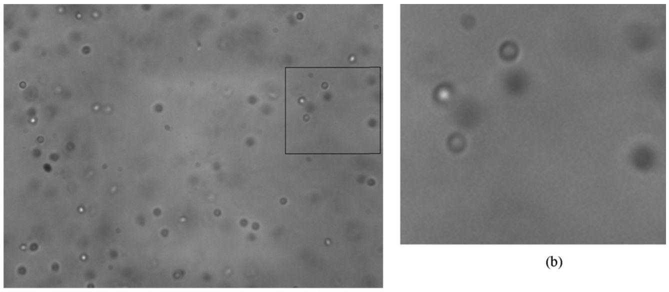

(a) (b)

Fig. 1. (a) Image of multiple polystyrene beads of Brownian motion. (b) Magnified region from (a) shows that some particles are seriously defocused.

approach to segment all particles of familiar shapes simultaneously and locate their center coordinates accurately. We propose an improved ACM approach that exploits the feature of boundary, shape and region of an image. The basic idea is to introduce a regularization term of shape prior, so that shape information of each object can be embedded into the energy functional. Therefore, the improved energy term can drive the active contour toward the shape prior gradually whatever the position of the active contour is in the image. For the numerical implementation, we use a two-step splitting method to iteratively solve the functional equations. Furthermore, the Hough transform (HT [18,19]) can automatically identify circular features in the image based on intensity gradient information. In this paper, combining with Hough transform, we utilize the contour line of each particle to locate centers of the detected particles.

Individual colloidal particle with diameter roughly larger than 200 nm can be observed with a conventional light microscope. In this paper, we utilize the suspension of polystyrene beads (4009 Å, ~1 μm diameter) to verify the validity of our method. We use an Olympus IX70 inverted microscope with an oil immersion objective (100×, N.A. 1.35, Japan) and a charge-coupled device (CCD) camera (Coolsnap Cf2, America) coupled to our microscope's video port to visually monitor the motion of particles. By digitizing video frames, we can acquire a sequence of digital images. In order to track the position of these beads and measure their motion trajectory, the first task is to segment the beads from the individual frame of the sequence. The rest of the paper is structured as follows. The related previous work about active contour models is reviewed in Section 2. Our novel integrated active contour method is presented in Section 3. The experimental design and analysis of the results are presented in Section 4. In Section 5, we present our concluding remarks and suggest further applications of our scheme.

2. Theoretical basis of the proposed method

2.1. Edge-based model

In the traditional edge-based active contour models, edge detectors are used to stop the evolving curve on the boundary of the desired object [12]. Usually, considering an image I, a positive, decreasing and regular edge-function $g(|\nabla I|)$ is selected, such that $\lim_{t\to\infty} g(t) = 0$. For instance

$$g(|\nabla I|) = \frac{1}{1 + |\nabla G_\sigma * I|^p}, \quad p \geq 1 \tag{1}$$

where $G_\sigma * I$ denotes a smooth version of I convolved with the Gaussian kernel G_σ with standard variance σ. The function $g(|\nabla I|)$ is strictly positive in homogeneous regions, and it is near zero on the edges. A typical geometric edge-based active contour model is given by the following evolution equation

$$\begin{cases} \dfrac{\partial \phi}{\partial t} = g(|\nabla I|)\left(\operatorname{div}\left(\dfrac{\nabla \phi}{|\nabla \phi|}\right) + \nu\right)|\nabla \phi| \\ \phi(0, x) = \phi_0(x) \end{cases} \tag{2}$$

where $g(|\nabla I|)$ is the edge-indicator function defined in Eq. (1) with $p = 2$, ν is a positive constant, and ϕ_0 is the initial level set function. Its zero level set curve moves in the normal direction with the velocity of $g(|\nabla I|)(\operatorname{div}(\nabla\phi/|\nabla\phi|) + \nu)$ and stops at the desired boundary, where g vanishes.

2.2. Region-based segmentation with level sets

Let $\Omega \subset R^N$, where $N = 2$ or 3 be the image domain and $I : \Omega \to R$ be an input image. Mumford and Shah [20] approximated an image with a piecewise-smooth function $u : \Omega \to R$, such that u varies smoothly within each sub-region and abruptly across the boundaries of the sub-regions. Let $C(p) : R \to \Omega$ approximates the edges of the sub-regions. The energy functional is given as follows:

$$E^{MS}(u, C) = \int_\Omega (I - u)^2 dx + \mu \int_{\Omega \setminus C} |\nabla u|^2 dx + \nu|C| \tag{3}$$

where $\mu > 0$, $\nu > 0$ are two fixed parameters, and $|C|$ represents the length of the contour. The image segmentation can be performed by minimizing Eq. (3) with respect to u and C. However, it is difficult to minimize the above functional in practice, due to the unknown set C of lower dimension and the non-convexity of the functional.

2.3. Shape-based model

In many applications of image segmentation, some prior knowledge about the shape of the expected objects is available. F_{shape} is a functional that depends on the active contour providing the boundaries. This functional evaluates the shape differences between the level set and the zero level set of the shape prior [21,22]. The level set formulation of the shape functional is given as

$$F_{shape} = \int_\Omega (\phi(\mathbf{x}) - \psi(\mathbf{x}))^2 |\nabla\phi| \delta(\phi) d\mathbf{x} \tag{4}$$

$$A(x, y) = \begin{pmatrix} A_x \\ A_y \end{pmatrix} = s \begin{pmatrix} \cos\theta & \sin\theta \\ -\sin\theta & \cos\theta \end{pmatrix} \begin{pmatrix} x \\ y \end{pmatrix} + \begin{pmatrix} T_x \\ T_y \end{pmatrix} \tag{5}$$
$$\forall (x, y) \in \Omega : s \cdot \psi(x, y) = \psi_0(A(x, y))$$

where $s > 0$ is a scale factor, θ is a rotational angle, and $T = (T_x, T_y)$ is the translation vector. Note that the selected representations [ψ, ψ_0] are invariant to translation and rotation. Thus, the transformation A creates pixel-wise intensity correspondences (level set values) between the current shape representation ϕ and the target shape ψ.

3. Our shape regularization ACM approach

In this section, we exploit the imaging features to develop an energy functional. For the application to polystyrene beads suspension, the segmentation should identify circle-like shapes and ignore inhomogeneous background fluid. As shown in Fig. 1, the differing of mean intensities is due to the defocus, overlap and background remnant. Therefore, segmentation via a single global active contour is insufficient. We construct the following energy functional to address the problem combining shape-based, boundary-based and region-based functional.

Our given particle image consisted of multiple objects $\{O_1, O_2, \ldots, O_n\}$ of familiar shape and we do not know how many