Path Planning for 3D Transportation of Biological Cells with Optical Tweezers

Tao Ju, Shuang Liu, Jie Yang, and Dong Sun

Abstract - **Manipulation of biological cells has recently drawn tremendous attention for its wide applications in biomedical fields such as cell-cell interaction, drug discovery, and tissue engineering. This paper presents a rapidly-exploring random trees (RRT) based path planner for transportation of biological cells in robotic transportation with optical tweezers in three dimensions (3D). By integrating the RRT algorithm into the optical tweezers manipulation system, we can successfully transport biological cells with high precision while avoiding obstacles during cell movement. Simulations and experiment are performed in transporting yeast cells to demonstrate the effectiveness of the proposed approach.**

Index Terms – Cell transportation, optical tweezers, 3D path planning.

I. INTRODUCTION

The last decade has witnessed tremendous growth in both research and applications of micro- and nano-scale biological tasks relevant to the health and well being of humans. Optical Tweezers has been widely used in the field of biomedical and physical applications in the last two decades. It has become a well-established technical tool to trap and manipulate small dielectric particles, which range from several nanometers up to tens of micrometers [1-2].

With optical tweezers, much effort has been extensively devoted to cell micromanipulation in many biological tasks such as cell transportation [3-4], cell sorting or isolation [5-6], experiment preparedness for cell injection [7-9], cell property characterization [10], cell-cell interaction [1], DNA stretching [11-12], and so on. Most of existing works in cell manipulation have been done in two dimensions (2D) plane, and very few of these approaches have been discussed in 3D space. The demonstrated capacity of optical tweezers for transporting biological cells in 3D is used to so many applications, including construction of crystals structures [13-14], assembly of reconfigurable microenvironments [15-16], and 3D configurations of a variety of different cell types in complex architectures [17].

Cell manipulation and property characterization integrated with robotics technologies have attracted increasing attention due to its high operation accuracy and efficiency. Using the forces exerted by a focused laser beam, optical tweezers can function as special robot end-effectors to trap and move biological cells. A few works have been done recently towards automatic cell manipulation [18-23] and cell characterization [9-10] by optical trapping. Most of the path planning methods for transportation of biological cells with optical tweezers are in 2D plane.

In this paper, we develop a new approach to cell transportation in 3D based on a RRT-based path planner. Path planning of macro robots has been extensively studied over the past decades [24-25]. RRT algorithm [26-28] has been investigated intensively in the fields of macro robot path planning. This algorithm can search collision-free paths rapidly without preprocessing, and does not need a grid-map representation, as in A* based path planning [3]. However, there is no report in the literature that RRT algorithm has been used in micro-scale task execution for path planning. In this study, a RRT-based path planner is proposed for the path planning of cell transportation. The path planning includes two steps: generation of a collision-free geometric path and path optimization. A unique significance of this paper is that we design a two-orthogonal observation system to extend the path planning from 2D to 3D space.

This is achieved by dividing a 3D cell transportation into two sub-transportations in the two orthogonal planes. The contribution of this paper lies in the proposal of using a robotic path planner to address the automatic transportation of biological cells in 3D with laser setup, and further improving the efficiency by determining the key waypoints of the generated path.

The reminder of this paper is organized as follows. In Section II, The RRT-based path planner is developed for automatic transportation of cells. In Section III, the two orthogonal observation system is established, and a 3D path planning for cell transportation is introduced. In Section IV, simulations and experiment are performed in transportation of yeast cells to demonstrate the effectiveness of the proposed approach. Finally, conclusion is given in Section V.

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II. RRT-BASED PATH PLANNER

A RRT-based path planner is developed first for the automatic cell transportation in 2D plane. Using the path planner, a collision-free path of the cell is planned after the cell is trapped by the optical tweezers and a collision-free goal position is selected.

The proposed RRT-based path planning includes two steps.

A. Generation of initial collision-free path

The first step aims to generate an initial collision-free geometric path by expanding a random tree T that is comprised of a series of vertexes. This step starts from initializing the tree T to its start state q_{init}, as shown in Fig. 1. Insert the initial state as the first vertex. Suppose the motion of the cell is governed by a control law, $q_{k+1} = f(q_k, u_k)$, $q_k \in C$, $u_k \in U$, where C denotes the configuration space, k denotes the index of the vertexes, and U denotes the distance space based on C. Select a random point $q_{random} \in C$, and find its nearest neighbor q_k in the tree, which has the shortest distance with the point q_{random} on C. Choose the control u_k that pulls the vertex q_k toward the point q_{random}. A new vertex is then generated for a new state $q_{k+1} = f(q_k, u_k)$, and a new edge e connecting the vertexes q_k and q_{k+1} is generated. If e lies in the free configuration space denoted by C_{free}, insert the new vertex q_{k+1} into the tree as a child of q_k, as shown in Fig. 1. To reach the specific goal position q_{goal} in the path planning, the proposed RRT path planner selects q_{random} bimodal: with probability p, choose the goal position q_{goal}; with probability 1-p, choose a point uniformly from the configuration space. If the tree reaches the goal q_{goal}, the initial collision-free path is generated; otherwise, the procedures are repeated.

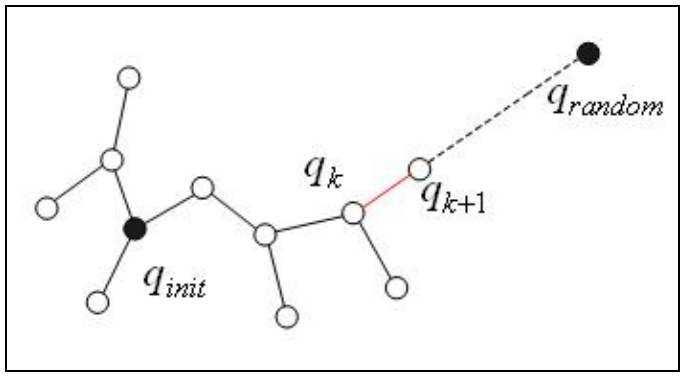


Fig.1 Growth of a RRT tree.

B. Path optimization

The second step is to optimize the generated path by shortening the path length. In the first step, numerous waypoints are generated in the geometric path, and if the cell is dragged along this path, the cell has to stop at too many waypoints, in which way the efficiency of the motion will be very low. In the second step, we will determine some important waypoints in directing the path, and discard the rest unnecessary waypoints, which will optimize the generated path and improve the efficiency of the cell's motion. We denoted these determined ones as key waypoints (KWPs), and the optimized path can be represented by these KWPs.

We design a KWP-pruning function to prune the initial geometric path and select the most important positions as KWPs. Define the initial geometric path l by a range of waypoints, denoted by $l = \{c_1, c_2, ..., c_k, ...\}$, where $c_1, c_2, ..., c_k, ...$ denotes the position of waypoints, and k denotes the index of the positions along the path starting from the initial position. The initial and the goal positions are defined as the first and the last KWPs, respectively. The rest position c_k is determined as a KWP, only if the straight-line connection of its two neighboring waypoints c_{k-1} and c_{k+1} is not collision-free. The connection between c_{k-1} and c_{k+1}, $k = 2,3,\ldots$, is checked in succession starting from c_2. The illustration of KWPs determination will be shown in the simulations of section IV. After path optimization using such KWPs determination, a shorter straight-line path that has much few waypoints will be generated. The efficiency of the cell's motion can thus be improved.

III. 3D PATH PLANNING

Generally, the trapped cell is transported on the focal plane of the optical microscope in the 2D space. To extend the cells manipulation to 3D space, 3D vision should be first constructed. We plan to develop a robot-aided optical tweezer setup for 3D cell manipulation, as shown in Fig. 2. An easily assembled disposable specimen chamber with a window for top and side view is designed, in a similar way to the sample chamber devised in [29]. To effectively remove the constraints on the sample chamber dimensions, we employ two low numerical aperture objectives with long working distances, which thereby gives a previous distance from objective lens to the trapping region. In both the transverse plane(x-y plane) and axial direction(y-z plane), the images of trapped cell are collected from CCD cameras, and the image data are obtained after image processing from the two orthogonal projections of the sample volume.

In a similar manner to [30], we design a feasible approach to cell transportation in 3D based on a RRT-based path planner. The path generation of a single cell in 3D space can be achieved by dividing the path plan into to plans in two orthogonal observation planes. When a collision-free goal position is selected in 3D, a collision-free subgoal position is calculated on the intersecting line between the x-y plane of the trapped cell and the y-z plane of goal position. The transportation of trapped cell to the goal position is then

decoupled into two sections: transportation between a start position and a subgoal position in x-y plane, and transportation between the subgoal position and a goal position in y-z plane. Based on the two images from two orthogonal CCD cameras, two paths will be generated on these two planes based on the RRT-based path planner in 2D space. As a result, the automatic transportation of biological cell in 3D space is converted into two transportations in 2D plane.

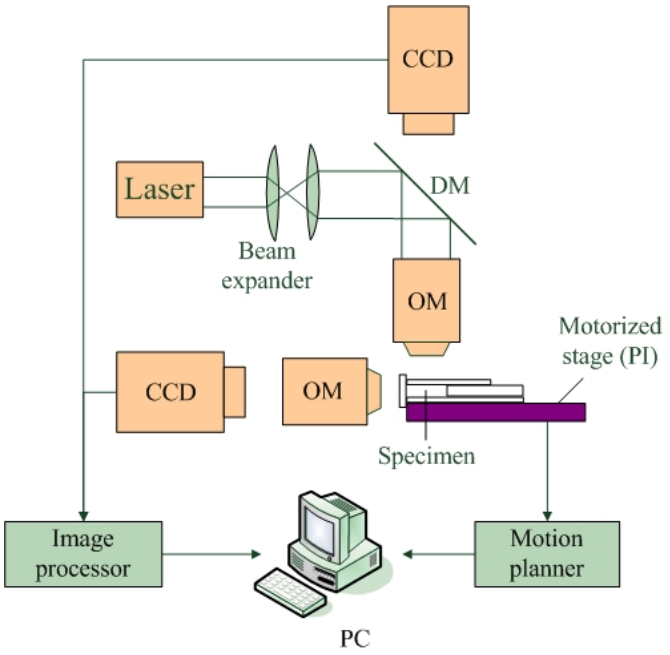


Fig .2 Robot-aided optical tweezer setup. The laser beam is passed into the microscope objective by means of a dichroic mirror (DM), which reflects the laser light. Then the beam is focused on the specimen that is precisely located and moved by the X-Y-Z stage. The fastened focused laser generates an optical tweezer to trap and manipulate biological cell suspended in a liquid environment. The relative movement between the trapped cell and the specimen is performed when the stage is motorized. Trapped cells are imaged with the objective onto two CCD cameras in two orthogonal observation planes.

A control software is developed in Visual C++ 6.0 and employed for automatic cell-transportation in 2D plane. The constructed modular architecture includes a graphical user interface (GUI) module, an image processing module, and a motorized stage execution control module. Fig. 3 illustrates a block diagram of the hierarchical control of the cell manipulation procedures. The trap position is obtained from the image processing module. In the motorized stage control module, a dynamic control is achieved by receiving a series of commands. In the cell transportation, a binary map is constructed based on the image obtained from the CCD camera, and recognized by the execution module. Based on the digitalized information of the binary map, a collision-free path from the trapped position to the goal position of target cell is generated by using the proposed RRT-based path planning algorithm. The X-Y stage is then motorized to move the target cell along the generated path composed by a series of KWPs. The generated path might be not available due to the variation of the micro environment. Hence, the path planner checks the validity of the following path when the cell arrives in the first KWP. If the following path is not available, a new path will be generated. In this manner, the trapped cell can be ultimately transported to the goal position.

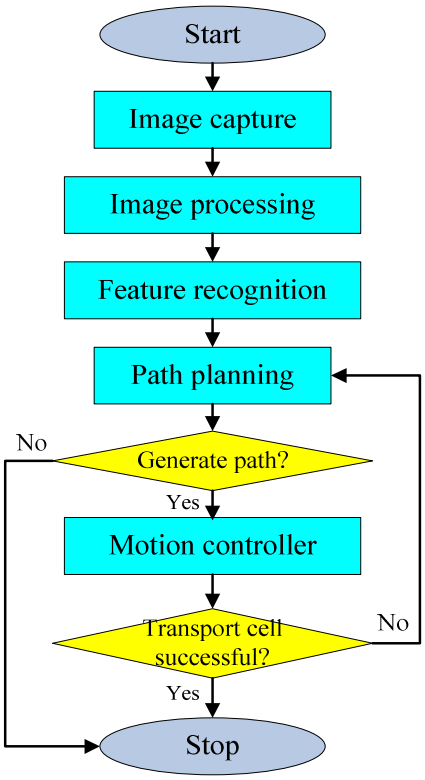


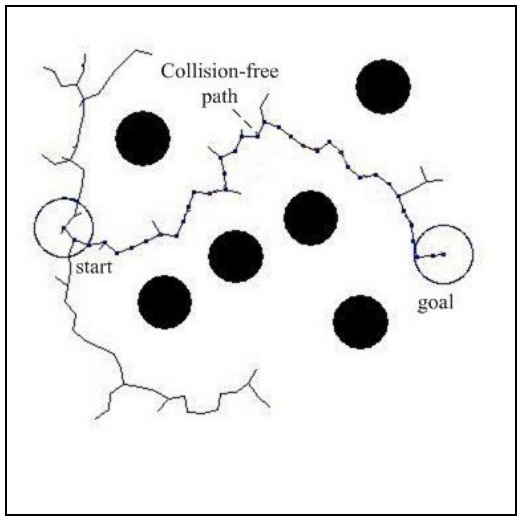
Fig. 3 Cell manipulation procedures with the single optical tweezer setup

It is worth noting that the focused laser beam is stationary, and the images collected from the two CCD cameras are fixed on the x-y plane and y-z plane at the focus distance of laser beam. The specimen is precisely fixed on the X-Y-Z stage, and the relative movement between the trapped cell and the specimen is obtained through motorizing the X-Y-Z stage.

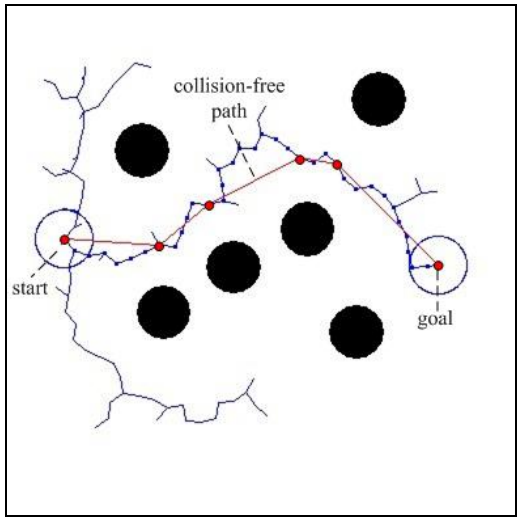
IV. Simulation and Experiment

A. Simulations in 2D and 3D world

To demonstrate the validity of the proposed approach for the 3D cell transportation, simulations in two orthogonal observation planes were performed in Microsoft Visual C++ 6.0 platform under Microsoft Windows XP. The environment is 400×400 (pels).



(a) Step 1



(b) Step 2

Fig. 4 x-y plane

The simulations were performed in both x-y plane and y-z plane. The path planner aimed to generate a collision-free path from the start position to the goal position, as shown in Fig. 4. By using the proposed RRT-based path planner, a rapid exploring tree was expanded from the start position until it reached the goal, and an initial path was then obtained, as shown in Fig. 4(a). The generated path was further optimized by the proposed KWPs determination, and a new collision-free path was generated, as shown in Fig. 4(b), in which the endpoints represent the KWPs. It is seen that the path length was greatly shortened and many unnecessary waypoints were removed. In the same manner, the collision-free path was generated in y-z plane, as shown in Fig. 5. As a result, a 3D cell transportation could be realized by sub-transportations in the two orthogonal planes.

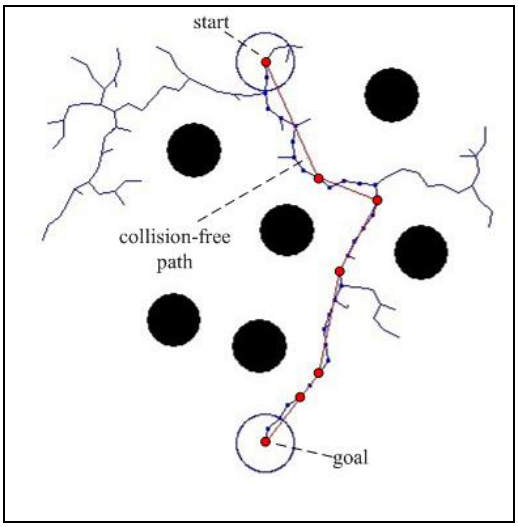


Fig. 5 y-z plan

Figure 6 illustrates the simulation of cell transportation in 3D space. The subgoal position is the goal position for the path planning in x-y plane and the start position for the path planning in y-z plane, which is chosen heuristically. The final collision-free path of cell transportation was made up in a 3D configuration.

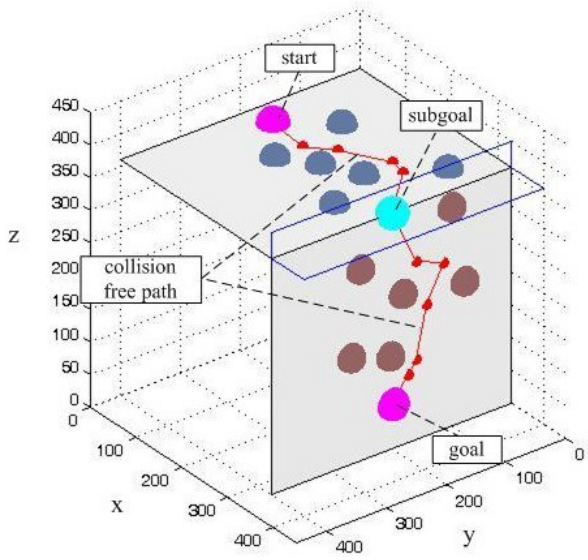


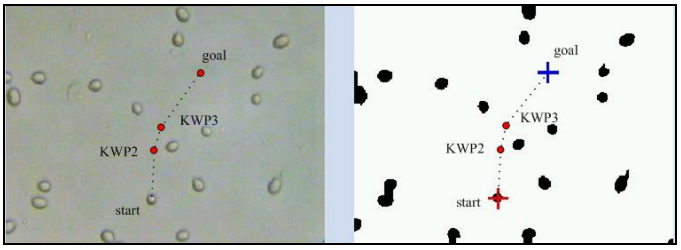
Fig. 6 Cell transportation in 3D world

The simulation results demonstrate that the proposed path planner could search a collision-free path for the cell manipulation successfully in 3D space, by dividing cell transportation into two sub-transportations in two orthogonal observation planes.

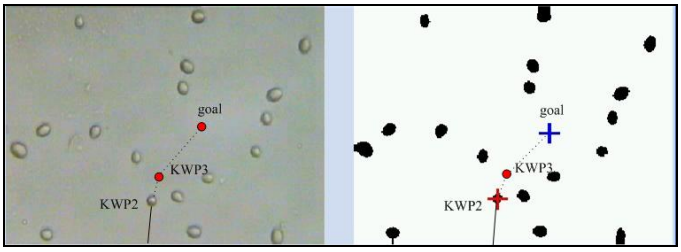
B. Experiment

Since the 3D cell manipulation platform is still under construction, we only reported the experiment of transporting a yeast cell with the optical tweezer system in 2D plane [4]. The experimental setup we constructed was installed on a

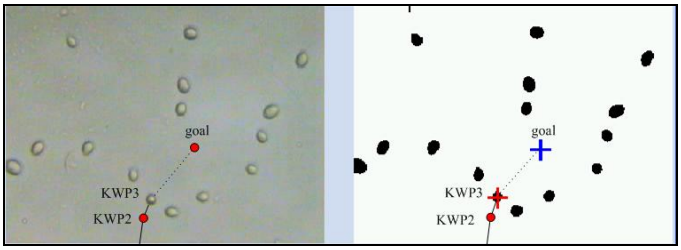
vibration isolation table, including a diode-pumped solid state laser generator (808nm, 2W), a microscope equipment with magnification ×40 , a CCD camera, and a X-Y computer-controlled motorized stage (PI M-111.1DG). The laser power was set as 700mW. The moving velocity of the x-y stage was about 3 $\mu m/s$. A single cell was manually selected and trapped at the start position, and was then transported to the goal position, as shown in Fig. 7(a). A few other yeast cells were regarded as obstacles in the environment. The left-hand side are the images from CCD and the right-hand side are the binary images after image process and path planning.



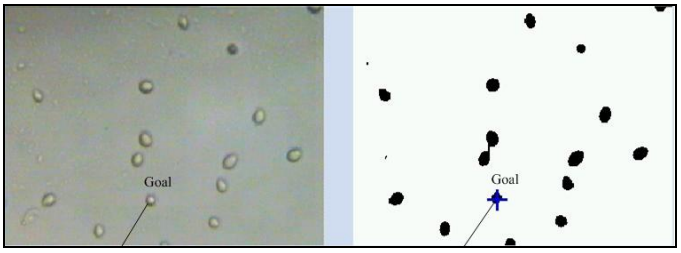
(a) The generated path with four KWP



(b) Move to the second KWP



(c) Move to the third KWP



(d) Move to the last KWP

Fig. 7 Cell transportation in 2D experiment

Using the proposed path planner, we obtained a collision-free path which composed four KWPs, as labelled in Fig. 7(a). The X-Y stage is then motorized to move the target cell along the generated path. The cell stopped and obtained the information of the next waypoint when it arrived at each waypoint along the path, as shown in Fig. 7(b-c). The solid lines represent the paths that have been passed by the cell, and the dotted lines represent the paths to follow. The target cell was successfully transported to the goal position in Fig. 7(d). Since the travelling environment might change due to the cells' Brownian movement, the path planner checked the validity of the following path at each way point and updated the path if necessary. The experiments demonstrate that the effectiveness of the proposed path planning approach in single cell transportation with the optical tweezers in the 2D microenvironment. A 3D transportation is an integration of two sub-transportations in 2D respectively.

V. Conclusion

In this paper, a robot-aided optical tweezers manipulation tool is proposed to address the automatic transportation of biological cell in 3D space. A RRT-based path planning method is developed to automatically transport a cell trapped by the optical tweezer. Simulations and experiments are performed on a yeast cell to demonstrate the effectiveness of the proposed approach. Our future work will focus on the construction of 3D cell manipulation setup and manipulation of multiple cells in a more dynamic environment in 3D space.

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