Tracking Multiple Particles in Confocal Microscopy

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Abstract #2

A system for tracking multiple nanometer-scale fluorescent particles in a confocal microscope and an experimental validation is described. Position estimates of an individual fluorescent particle are generated from fluorescence intensity measurements taken at a small number of discrete locations. Tracking is achieved by combining the estimation procedure with a linear quadratic Gaussian (LQG) regulator. Multiple particles are tracked by combining the models for individual particles into a single system, applying the same LQG framework, and then cycling the control through each subsystem in turn. Experimental results are presented for single and multiple particles. For validation purposes, during each experiment images from a charge-coupled device camera were captured and analyzed offline using a standard Gaussian fit method. The estimated trajectories were in good agreement with those produced by the LQG algorithm, thereby verifying the tracking scheme.

Introduction

Tracking fluorescent particles in confocal microscopy holds great promise for the study of processes inside living cells. Most current techniques to detect and track fluorescent particles rely on either the wide-field fluorescent images captured by a CCD camera [1], or tracking in single/multi photon microscopes [2,3]. Tracking fluorescent particles in fluorescence microscopy holds great promise for the study of processes inside living cells. Most current techniques to detect and track fluorescent particles rely on either the wide-field fluorescent images captured by a CCD camera [1], or tracking in single/multi photon microscopes [2,3].

Position Estimation Algorithm

The point spread function (PSF) of a diffraction-limited spot can be approximated and modeled by a Gaussian function given by a CCD camera [1], or tracking in single/multi photon microscopes [2,3].

Experimental System Setup

Confocal tracking microscope: Fluorescence is excited in the sample using a laser beam focused to a diffraction-limited spot through the objective lens. The output fluorescence is collected by the objective and separated from the laser using a dichroic mirror. This signal is then passed through a beamsplitter, sending a fraction R to a CCD camera and the remainder to an APD.

Experimental Results

The tracking capabability of the system is mainly limited by the scanning speed and positioning accuracy of the nanopositioning stage which restricts us to a finger-to-finger control. A new control algorithm is described below to drive a second-order nanopositioning stage from an initial to final position with zero initial and final speed. Moreover, the online final time estimate makes it feasible to optimize the resulting trajectory over a collection of positions to minimize the total travel time.

Time-Optimal Control

The tracking capability of the system is mainly limited by the scanning speed and positioning accuracy of the nanopositioning stage which restricts us to a finger-to-finger control. A new control algorithm is described below to drive a second-order nanopositioning stage from an initial to final position with zero initial and final speed. Moreover, the online final time estimate makes it feasible to optimize the resulting trajectory over a collection of positions to minimize the total travel time.

Conclusions and Future Work

We have presented a scheme for tracking nanometer-size fluorescent particles that combines a position estimation algorithm with an LQG controller. We have validated the tracking capability in two dimensions through experiment. Future work will focus on the extension to 3-D tracking by using the fluorobenzylfluoride algorithm for localization in three dimensions [4] and the improvement of the system by increasing the speed of hardware and controller development to enable the ability to rapidly and accurately move the relatively large distances between particles.

References


The author greatly acknowledges the support of the NSF through IDBR Program Grant No. DBI-0649823.