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A Web Interface for the Quantification of Microtubule Dynamics

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Abstract

We propose a web interface that allows researchers to quantify and analyze microtubule confocal images online. Most analyses of microtubule confocal images are performed manually using very simple software or tools. Analysis results are stored locally within each collaborator with different styles and formats. This has limited the sharing of data and results when collaborating among different research parties. A web interface provides a simple way for users to process data online. It also allows easy sharing of both data and results among different participating groups. Analysis workflow of the interface is made similar to existing manual protocols. We demonstrate the integration of image processing algorithm in the current workflow to aid the analysis. Our design also allows integration of novel automated analysis algorithms and modules to re-evaluate existing data. This interface can provide a validation platform for new automated algorithm and allow collaboration on microtubule image analysis from different locations.

SECTION 1. Introduction

Microtubule has become a target of cancer chemotherapy since it is responsible for many functions in a cell. A popular choice for analyzing and quantifying microtubule response to chemotherapy treatments is to use confocal microscopy images. Specifically, microtubule dynamics are measured under different experiment conditions. Microtubule dynamics refer to the stochastic nature of microtubule movement where microtubules are observed to switch between periods of growing, pausing, and shortening [1]. The activities of microtubules are related to many cell functions including cell division and cell movement. While the effect of high level of Taxol dose on microtubule is known, the effect of low level dose is not as clear. Understanding the effect of low level of Taxol on microtubule allows better design and application of Taxol as a chemotherapy treatment for cancer.

Microtubule dynamics are typically imaged using confocal fluorescence microscopy. Green fluorescence protein (GFP) is used to improve the image contrast. For imaging microtubules, GFP-tagged tubulin is used. Imaging the dynamics of microtubule requires a time-lapse

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capturing of microtubule. A series of images of the same cell is captured to see how the morphology of the microtubule assemble changes over time. The length of the time under observation depends on the photo-bleaching effect of the GFP as well as the time between successive images. How often an image is captured would depend on the exposure time and the acquisition time of the camera employed. Experiments can range from several minutes to several days.

After images are captured, the analysis of microtubule dynamics involves measuring the movement of microtubules through time. Currently, the length of microtubule is measured from the images. The length of a microtubule is defined as the distance from the plus-end of a microtubule to a reference point along the selected microtubule body. Analysis is done manually by defining the reference point in the first frame of the image series, and locating the different locations of the moving microtubule plus-end in every frame in the series. Length data from different microtubules are collected for a particular experiment condition to derive the population statistic that would characterize that particular experiment condition. Microtubule statistics are commonly expressed in term of the parameters for microtubule dynamics that includes average microtubule velocities, time spent in growing, time spent in shrinking, rescue frequency, etc. Microtubule life-history plot is used to show the changes in length versus time.

While manual analysis protocol has been established for the microtubule experiments, the implementation of the protocol usually involves locally installed software, provided by the microscopy manufacturer or as freeware like ImageJ [2]. Communicating these manually analyzed results to other parties is difficult since the format of the analyzed result depends on the protocol defined by the original party that analyzes the data. Some computer algorithms have been developed to automate the image analysis process [3][4][5][6]. Most of the software is local installation and many do not address how the results are being stored and managed. The MTrackJ plugin [7] for ImageJ provides an automated analysis algorithm integrated with existing software platforms, but it is limited to the analysis of microtubules imaged in a different imaging methodology. Making the microtubule image analysis system accessible online becomes an attractive way to improve the communication between different collaborating parties. An online interface brings little changes to existing manual analysis protocol and it allows the data to be stored and shared to other collaborating parties. It also aids the development of automated algorithm as it provides a database of ground truth for algorithm evaluation and validation. Feedback from the automated analysis can provide information on how to design future experiments and image acquisitions.

We propose to make a web interface that replicate current manual analysis work flow of microtubule images [8]. Our design uses a web server and a data server that is located in separated networks. The system has the capability to integrate other automated microtubule analysis algorithms. In this paper, we show how image processing algorithm can be integrated into the existing workflow to improve the current analysis protocol. We also show the results of the image analysis shown as the parameter of microtubule dynamics indicating the population statistics and as microtubule life-history plot to show the length change versus time.

SECTION 2. Methods

The proposed system provides a web interface for the image analysis of microtubule. Existing protocol for manual image analysis is followed. It also allows the integration of image processing algorithm implemented in Matlab with online accessibility. Management of image data and visualization of analysis results can also be accomplished over the web.

2.1. System Design

Figure 1 shows the design diagram for the web interface system. All user activities are performed in the web server. We use Apache for the web service and PHP to process web page data. Data provided by the users as well as other annotation data are captured from the web server and sent to the data server that performs the image processing. MySQL is used as the database for the system. All data are processed using Matlab scripts stored in the data server. Both raw and processed images are stored in the data server. After the images are processed, result images are returned to the web server for display. All the annotations, information about the microtubule image (image acquisition details, experiment information), and the parameter settings for image enhancement are stored in a database for data management purposes.

Currently the web server and the data server reside in different networks. While the two servers can be located within the same network, they are separated due to existing infrastructure. However, it also results in a more modular design that allows for easier upgrade of individual components. All original image data are stored in the data server. Compressed and down-sampled images are sent to web server for display to save bandwidth and hard disk storage. We use Matlab as the image processing software but it can be easily converted to accept algorithms implemented in other languages as well by generating the appropriate scripts to send to the data server.

2.2. Workflow

A typical workflow for quantifying and analyzing microtubule using the web interface is shown in Figure 2. When a user first accesses the page, the users can choose between uploading new data or manage existing data that had been uploaded previously. After that, the web interface allows uses to choose between

1. image enhancement,
2. image analysis and viewing,
3. viewing analyzed microtubule statistics.

Microtubule data are time series data, and the data size is often too large for real-time processing. Hence for image enhancement, the process is performed in a batched fashion and an extra update process step is needed to see whether or not the image enhancement process is completed. Each image enhancement is submitted as processing job to the data server and completed process will be reflected when updating the list of data in the interface. Enhancement can be repeated with different parameters so the best image can be obtained. The analysis of microtubule image can be performed with or without using the enhanced

image. Once the image data is analyzed, the results can be viewed as an image with the microtubule marked or results from multiple microtubules are grouped together and their aggregate statistics are displayed using microtubule dynamics parameters and life-history plot.

Since the main potential users of the algorithms are researchers that perform the experiments, the interface is kept simple and performs only the essential steps during the manual analysis workflow.

2.3. Database Design

A database is also developed for record keeping. Figure 3 shows the relationship between different tables. Five tables are used in the database design. The experiment table records details about the condition which the microtubules are being imaged. This includes the drug name used, time between application of drug and imaging of cell, imaging equipment properties, and information of imaging protocol. Since confocal imaging allows capturing of images across different focal planes on the z-axis, each focal plane would result in a series of images after the capturing process. These images are grouped under the table called video_frame. It includes information about the location of the focal plane as well as various flag variables to track the progress of image processing and microtubule analysis. The image table stores relevant data about each particular image including such as the time point along the sequence, location of the focal plane along the z-axis, and parameters used in image enhancement. Segmented microtubule is also stored in the database, a table called microtubule is created to keep track on which image sequence a segmented microtubule is belong to. Individual points on the segmented microtubule are stored in the microtubule_point table. Information, for example, the x and y coordinate of the each point along the microtubule, is recorded so that it can be visualized by overlaying it on top of the microtubule image.

2.4. Web and Data Server Communication

Data link between the web server and data server is established using ssh. Scripts in the web server provide the automated login for various functions, for example, uploading files and executing Matlab function on the data server. Since image processing uses Matlab functions, Simple Matlab script is generated in web server as well where it stores some of the variable initializations and parameter settings for the Matlab functions stored in the data server. These simple scripts are generated and uploaded to the data server when users request the processing in webpage. Since the time needed for each image processing task may vary due to the size of the dataset, the web server will not check the completion of each task at the time of creation. Instead, a separated step to check the status of each processing job is created. The web server will check if a processing task is completed at the data server by checking the existence of the output file created by the data server. Once the processing task is completed, all necessary information is transferred back to the web server. Processed image, both compressed and down-sampled, are stored for display; and the database is updated accordingly.

2.5. Image Enhancement and Quantification

For image processing, it is divided into image enhancement and microtubule analysis. Matched filtering is used for image enhancement. It is designed specifically to enhance the curvilinear structure that microtubule resembles. A bank of filter templates H_i that models a short segment of microtubule at different orientations is created. These templates are used to filter the input image I and the best result is retained to form the enhanced image F .

$$F(x, y) = \max_i \sum_{u, v} I(u, v) * H_i(\sigma, \theta_1) \quad (1)$$

Users are able to adjust the number of templates (i.e. orientations, θ_i), and the width σ , of the line segment in the templates as the parameter in the image enhancement page. For image quantification, users are able to go to each image and select microtubule from either the original or the enhanced image for quantification. Quantifying a microtubule requires users to click on the image and all the clicked points are saved in the database for later processing. The quantification process is the same as the existing analysis protocol of microtubule dynamics. Users select points that trace the microtubule body as well as the microtubule plus-end.

2.6. Result Visualization

Two ways to visualize the quantification results are provided in the interface. First the segmented microtubules can be shown on top of the microtubule image to show the location and shape of the analyzed microtubule. Second, multiple microtubule can be selected from the database and the statistics from the aggregated microtubule is displayed. The aggregated statistics includes microtubule dynamics parameters defined in literature including:

1. average microtubule velocity,
2. average growing velocity,
3. average shrinking velocity,
4. percentage time spent in growing,
5. percentage time spent in shrinking,
6. percentage time spent in pausing,
7. catastrophe frequency,
8. rescue frequency,
9. dynamicity.

A life-history plot of the selected microtubule is also shown along side with the aggregate statistics for visualization. The selection of microtubule is done in the data management page. Microtubules can be included in the aggregated statistics by selecting the checkbox next to the data listed in the management page.

SECTION 3. Results

We illustrate the current image analysis workflow with a series of screen captures of the web interface. Before starting the analysis and visualizing the results, input images are first uploaded using the web interface. The interface accepts a single zip file that contains all the microtubule images. In the example shown below, we use 10 sample datasets each with between 9 to 23 images in the each data set. For individual dataset, the image files are sequentially named corresponding to different time points during the experiment. Most acquisition software would perform this task, so the only work is to provide the zip file to the interface. After images are uploaded, we perform the image enhancement on all the images uploaded using 20 matched filters with the size of the filter set to 11. After the enhancement is completed manual analysis is performed on the 10 dataset by tracking the movement of one microtubule in each dataset. All results figures are generated base on the result of these data.

Figure 4 shows the result of image enhancement and the analysis page in the interface. The enhancement process removes the clutter shown as the haze in the image and enhances the microtubule body in the images. User is able to switch between original and enhanced version of the image and use the best image for quantification. Quantification of microtubule can be performed by clicking on the desired microtubule in the image shown on the webpage in a manner than is exactly the same as one would do during manual analysis. The x and y coordinate points of the analyzed microtubule will be stored in the database for future display and calculation of microtubule statistics. Result from the manual analysis will be shown as a line segment overlay on top of the shown image (left).

Users first select a reference point for the microtubule in the first frame, after that, only the microtubule tip is needed to be selected for the rest of the data.

Figure 5 shows the list of data that have been uploaded to the database. It allows users an overall view of the data available in the database, and lists other experiment-related information about the images. If there is an enhancement process being performed on an image series, an update link is created so users can check if the process has been completed. Also whether a particular data has been analyzed is also shown under the “analyzed” column. In this page, users can choose to view any particular data to see the result of image enhancement as well as the result of quantification. Aggregate microtubule statistics can be viewed by selected the desired set of microtubules in this page for display.

The time it takes to perform image enhancement depends on the size of the data. Analysis of the microtubule data can start without the enhance process being completed. Image enhancement is used as an additional option to the manual analysis process but it is not a required step.

Figure 6 shows the aggregate statistics from the selected microtubules from page in Figure 5. We also allow users to select a set of data from the experiment and view the statistics of the selection set. Typical microtubule dynamic parameters like average velocity, percentage time spent in growing, shrinking, and pause are displayed. Life-history plot of the selected microtubule is also shown by creating a SVG plot graph dynamically.

The microtubule life-history plot uses 10 colors to display different microtubules. It cycles back to the same color when displaying more than 10 microtubule histories. It becomes cluttered if too many microtubules are shown at the same time. Note that the range of the plot depends on the range of the data being plotted.

Note that pixel is used for length measurement so only data from similar experiment should be grouped to calculate the aggregate statistics because the physical size of the pixel between different experiments could be different. The mean and standard deviation of each category will be calculated and displayed in the table.

SECTION 4. Discussion and Future Works

We propose and design an online microtubule analysis interface to quantify microtubule images. This provides an easy-to-use interface for researcher to access, manage, and analyze microtubule confocal images. The interface is designed to replicate current manual analysis so using the interface will be familiar to existing analysis protocol. We use a separated data server to process users-provided data so special image processing algorithm can be integrated into the analysis workflow. Image enhancement using matched filter is added as an optional aid to the manual analysis. Traditional image analysis process can now be performed on the web interface exactly like it would if using locally installed software as what it has been done before. Analyzed data can be viewed as the segmented microtubule on top of the image. The interface can generate microtubule life-history plot and calculate parameters for microtubule dynamics based on the data selected by the users. The system process input image in the data server using Matlab scripts, this allows the update of image processing algorithm without drastically change the architecture of the interface components. Integration of new algorithms is simple since it requires only additional scripts to be generated. It is also adaptable to codes implemented in other language by only changing the scripts in the data server. The gathering of manual analysis data in a single database accessible on the web allow for easy collaboration of both experiment data and analysis result for different parties. This collected data can also serve as the ground truth for the development of automated analysis algorithms. Validation of new computer algorithm is made easier since expert in manual analysis can also evaluate the result from the automated analysis. Hence incorporating automated analysis algorithm is the work for immediate future. Other future expansion of features and functionalities include better result visualization and enhanced security of uploaded data.

SECTION 5. Acknowledgement

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Fig1.
System design diagram

Fig2.

Interface workflow Clear boxes indicates users commands, shade boxes indicates information generated by the web interface

Fig3.
Database design

Fig4.
Image enhancement and analysis Left: Original image; Right: Enhanced image

File Name	Drug Name	Dosage (nMol)	Duration (frames)	Application point (hours)	Pre-processed	Analyzed	Layer #1	Update Status
☐ ADAMDCR_488nm_20002.zip	Control	0	40	10	Completed	Completed	New data	
☐ test01.zip	Control	0	40	10	Processing	Completed	New data	Update
☐ ADAMDCR_488nm_20000.zip	Control	0	40	10	Processing	No	New data	Update
☐ test02.zip	Control	0	40	10	Completed	Completed	New data	
☐ MT0309_0501.zip	Control	0	40	10	Completed	No	New data	
☐ MT0309_0502.zip	Control	0	40	10	Completed	No	New data	
☐ MT0309_0503.zip	Control	0	40	10	Completed	No	New data	
☐ MT0309_0504.zip	Control	0	40	10	Completed	No	New data	
☐ MT0309_0505.zip	Control	0	40	10	Completed	No	New data	
☐ MT0309_1901.zip	Control	0	40	10	Completed	No	New data	
☐ MT0309_1902.zip	Control	0	40	10	Completed	No	New data	
☐ MT0309_1903.zip	Control	0	40	10	Completed	No	New data	
☐ MT0309_1904.zip	Control	0	40	10	Completed	No	New data	
☐ MT0309_1905.zip	Control	0	40	10	Completed	No	New data	

Select boxes to show statistics

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Fig5.
Data management

Fig6.
Layout of the result visualization page

Statistics from Manual Analysis	Value	Unit
Number of Microtubules	10	N/A
Average Length	70.03±25.23	Pixels
Average Velocity	0.11±1.27	Pixels/Frames
Average Growing Velocity	5.49±2.78	Pixels/Frames
Average Shrinking Velocity	7.63±4.96	Pixels/Frames
Percentage Time Spent in Growing	11.76±0.07	%
Percentage Time Spent in Pausing	78.92±0.18	%
Percentage Time Spent in Shrinking	9.31±0.1	%
Catastrophe Frequency	0.04±0.13	Events/Frames
Rescue Frequency	0.04±0.17	Events/Frames
Dynamicity	1.36±1.16	Pixels/Frames

Fig7.
Detail view of the microtubule statistics table