IPAD FOR BIOIMAGE INFORMATICS

by

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(Under the Direction of Tianming Liu)

ABSTRACT

Microscopic bioimaging has become a critical approach in the analysis of image-based phenotypes in a variety of biological studies. Driven by these applications, computerized analysis and management of bioimages have been actively studied in recent years. However, there has been very little research effort devoted to the design and development of better graphical user interface (GUI) specifically for average biologists. Recently, Apple designed and marketed the iPad tablet computer as a general platform for consumer media such as book, movie, music, game, and web content. Using the state-of-the-art multi-touch technology on the touch-screen display of iPad has revolutionized user's experience in computer-human interaction. As a consequence, applications of iPad in bioimaging informatics are on the horizon. This thesis presents our initial effort in using iPad as a general platform for bioimage informatics in the applications of neurite tracing, zebrafish segmentation, cell segmentation, somite annotation and mobile bioimage informatics.

INDEX WORDS: iPad, interactive bioimage segmentation, cell segmentation, bioimage annotation, visualization, bioimage management, mobile bioimage informatics.

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B.E., Northwestern Polytechnic University, CHINA, 2007

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment

of the Requirements of the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2011

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May 2011

DEDICATION

To my mother and father,

without whose encouragement and inspiration

this thesis would never have been written.

ACKNOWLEDGEMENTS

I would never be able to finish my thesis without the guidance of my committee members and support from my friends.

I would particularly like to express my deepest gratitude to my advisor, Dr. Tianming Liu, for all his excellent teaching and providing me with an excellent atmosphere for doing this research. And I also would like to thank Dr. Kang Li and Dr. Suchendra M. Bhandarkar for their patient suggestions. Finally I would like to thank my friends for supporting me.

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CHAPTER 1

INTRODUCTION

In recent years, microscopic bioimaging has played an increasingly vital role in many biological studies including pathway analysis, target identification, drug discovery, and systems biology [1-4]. Driven by these growing applications, computerized analysis of bioimages has been actively studied in the literature. A variety of commercial and academic bioimage informatics software tools are available [e.g., 5-16]. Also, there have been many academic research labs (e.g., [17-27]) that have contributed significantly to the development and distribution of free bioimage computing tools, which have significantly enriched the resources available to average biologists for bioimage analysis, as described in a recent review article [28].

Although current bioimage informatics tools are reasonably good for experienced biologists when performing standard bioimage processing and management tasks, the accompanying user interface for average biologists performing application-centered bioimage data management need significant improvements. In the field of computer science, the topic of graphical user interface (GUI) design has been extensively investigated, in order to provide effective user interfaces for generic or specific user groups. But there has been very little research effort devoted to the design and development of better GUIs specifically for average biologists in the bioimage informatics field. In reality, biologists and biological researchers have rapidly growing needs for improved GUIs for the analysis and management of bioimaging dataset. Their needs include: 1) ease of use: the software should be accessible and usable to average biologists

who might not have extensive computer skills. 2) ease of learning: the software should be self-evident with a manageable learning curve; 3) effectiveness and efficiency: the software should be highly effective and efficient in solving bioimaging problems, e.g., segmentation and annotation of biological image objects.

Also, current bioimage informatics tools are mainly based on desktop computers or for portable purpose on laptop computers with keyboard and mouse to use. Sometimes, it is hard to draw the outline of a foreground in an image. That is, because users need to put their index finger on the button of the mouse and keep pressing all the time during drawing until they finish drawing a contour. With interrupted or one small unclick, the contour may be not as the same as users want. Besides, keeping pressing index finger on the left click of the mouse may result in physical discomfort for users. Therefore, it is difficult for researchers to concentrate on the biomedical image data. Because ancillary devices like keyboard and mouse lack the flexibility [29] when delineating images, we believe that just moving fingers would be an accurate and fast delineating method in a free manner.

In general, bioimaging informatics applications are very diverse. Differences in the underlying biological questions, species, anatomical organs, cell types, imaging equipments, or imaging parameters all contribute to the diversity of bioimage informatics. Due to this tremendous diversity, there is no single algorithm or computational tool that can be used to solve all bioimage informatics problems. Therefore, development of effective GUI that allows biological researchers to interactively participate in bioimage informatics, including interactive segmentation, manual

tracing, semantic annotation, and fine-tuning parameters and settings, is crucial to meet diverse bioimaging informatics needs.

With iPad, which is a large, high-resolution, and multi-touch screen portable internet-accessing device designed and made by Apple Company, we found it would be a great interactive tool for bioimage informatics. The reason behind this choice is that multi-touch technique tool will provide an effective, efficient way for users when using their fingers to input the bioimage information, and the portable internet access feature provides convenience for users in anywhere with a network access.

Since its release in April 2010, iPad has gained increasing interest and popularity. Its applications in the field of biomedical and health sciences are emerging. In our view, there are two prominent attributes of iPad, among many other features, that presenting it as an attractive platform for biomedical image computing and management as follows. 1) Ubiquitous connection and portability. The iPad platform uses a seamless integration of Wi-Fi and 3G wireless, offering fast data speed over 3G cellular networks around the world. The iPad platform can seamlessly switch between 3G and Wi-Fi, ensuring users always have the best possible connection. Therefore, the iPad platform provides biomedical professionals with the ability to immediately access, view and manage biomedical images. Besides, the weight (around 1.5 pounds) and size (between smartphones and laptops) of the iPad platform make it a very portable device. The prominent characteristics of portability and ubiquitous connection make iPad an ideal general platform for biomedical image management. 2) User-friendly interface. The touch-screen display of iPad uses state-of-the-art multi-touch technology that is able to simultaneously register two or

more distinct positions of input touches [30]. For instance, when a finger touches and moves on the display surface, the finger trajectory can be recorded for later interpretation such as delineation, annotation, or segmentation. This natural and user-friendly interface is a very attractive feature for numerous users, partly explaining the popularity of iPad.

Due to the abovementioned advantages of iPad, it is increasingly gaining popularity in biomedical domains as a generic communication, visualization, and computing device. For instance, the FDA recently approved the first application that clinical physicians can use to view medical images and make diagnoses using an iPad [31]. The FDA reviewed performance test results on various portable devices and measured luminance, image quality, and noise in accordance with international standards and guidelines. In addition, the FDA reviewed results from studies with qualified radiologists under different lighting conditions, and concluded that the iPad was sufficient for diagnostic image interpretation under recommended lighting conditions.

This thesis focuses on the investigation of the feasibility of using iPad as a platform for interactive bioimage informatics. In particular, on bioimage segmentation part, this platform generally defines the boundary of biomedical objects in images and it is the prerequisite of many following analysis and quantification applications. However, due to the tremendous diversity of biomedical imaging settings and application requirement [28], it is sometimes very difficult to automatically perform segmentation in biomedical images. Hence, semi-automatic segmentation is a trade-off choice between accuracy and automation [32]. In addition, semi-automatically segmented images can be used for learning statistical models that could be potentially used to

facilitate automated image segmentation procedure [33]. The multi-touch interface in iPad provides an ideal platform for interactive image segmentation. In this thesis, we investigated two prototype systems for interactive bioimage informatics: 1) Mobile bioimage informatics. We use iPad as a front-end for user interaction and perform supervised segmentation at the back-end server. This prototype system aims to demonstrate the feasibility of integrating the user-friendly interface on iPad and powerful computing capability at the server end. 2) Biological object tracing and segmentation. We use iPad for biomedical object boundary tracing. We investigated five bioimage informatics applications including brain MRI image tracing, neurite tracing, zebrafish segmentation, cell segmentation, and somite annotation. This prototype system aims to demonstrate that it is more effective and accurate to perform manual image tracing, annotation, and segmentation using finger tracing on iPad than traditional manual segmentation using mouse tracing on desktop computers. Our preliminary studies and comparisons suggest that iPad is a powerful and effective platform for bioimage informatics. For instance, manual tracing of a biological object by finger on iPad take 30%-40% time less than manual tracing by mouse on traditional desktop computer. Meanwhile, tracing accuracy by finger on iPad also outperforms mouse tracing on desktop computer.

CHAPTER 2

TOUCH TECHNOLOGY in iPad

Touch technology

There are several touch screen technologies, such as Resistive, capacitive, infrared, optical imaging, dispersive signal technology and acoustic pulse recognition, in which the resistive and capacitive systems are mainly used in portable devices.

The resistive system has one normal glass panel which is coated with a conductive layer and a resistive layer, and a protect layer is put on the top of it to avoid scratches. Electrical current runs through those two layers when monitoring. When the user touches the screen, the pressure makes those two layers connect and a difference voltage of the current position will be captured, and then the touch point coordinates position will be calculated out. That means the user may use fingers, nail, stylus and other pointy objects to conduct a touch event. The resistive touch screen technology works only by applying pressure on screen, and it has slowly responsive action than the capacitive one has [34].

The capacitive screen uses a capacitive material layer, which is placed on a glass panel of monitor, to hold electrical charge. By touching the screen with a finger, some of that charge will be diverted to finger. The changes of the amount of charge at a specific point of contact will trigger calculation of relative differences in charge at each corner in order to obtain the exact touch coordinates position. The capacitive screen allows registering more than one touch event

6

simultaneously. Using capacitive screen, all touches can be identified by the system immediately and it is generally more smooth and responsive than the resistive touch screen which needs pressure perceived by the system. [34]

Touch in iPad

Although the touch screen technology is not new, the iPad's touch user interface technology is a milestone for touching electronics. In particular, the multi-touch technology is one of iPad's major innovations. Without multi-touch technology, typical touch system can only recognize a single focus, meaning that when the user touches the screen with two fingers at same time, the system can only recognize one point. Instead, the iPad multi-touch technology is capable of identifying two or more focuses at the same time. For instance, the user can zoom in or zoom out an image in operation by two fingers. The iPad multi-touch screen can respond to both touch points and their movements simultaneously. Essentially, the multi-touch technology provides a novel and effective approach that allows users to communicate their intention to application software. Importantly, for bioimage informatics applications, the multi-touch system implements direct finger manipulation in the simplest way. It sends user intention and touch events to the touch screen directly. This is exactly the implementation of the general principle of "what you get is what you see" in the user interface design field.

In general, the touches represent the heart of iOS interaction which is the Apple mobile operation system. Touches provide the most important and convenient way of communication of

users' intent to an application. Touches are not limited to button presses and keyboard interaction, but also including any designs and applications that work directly with users' taps and other gestures.

Specifically, finger touches on iPad carry the following information: where the touch takes place (both current and previous locations), what phase of the touch is used (e.g., finger down, finger move, and finger up corresponding to mouse down, mouse move, and mouse up in the desktop scenarios respectively), a tap count (e.g., single-tap/double-tap), and when the touch takes place (via a time stamp). Apple provides UITouch objects in its development environment Xcode, which are used to store the touches and the related information. Each touch event corresponds to an object [35].

In touch events, there are different life cycles and phases that represent the progress of the touch within an interface. Each touch event can transit to any of the five phases. These phases are listed as follows [36]:

➢   UITouchPhaseBegan: means the user starts to touch the screen.

➢   UITouchPhaseMoved: stands for a touch moved on the screen.

➢   UITouchPhaseStationary: indicates that a touch stops moving on the screen surface but still holds on the screen surface.

➢   UITouchPhaseEnded: means the user stops touching the screen surface and pulls away the finger from the screen.

➢   UITouchPhaseCancelled: states that the iOS (iPhone or iPad Operating System) does not continuing tracking a particular touch.

Taken as a whole, these five phases define the interaction methods for a touch event. They fully explain all the possible ways that a touch can progress or fail to progress within an interface and also represent the basis controls for that interface.

All the five phases mentioned above need pre-defined callback methods to respond. The pre-defined callback methods take actions of the start, movement, and release of touches from the screen. Corresponding to the phases mentioned above, the methods involved are as follows. [37]

- ➢ touchesBegan:withEvent:— be called at the beginning phase of the event, when the user initially touch the screen.

- ➢ touchesMoved:withEvent:— handles the movement of fingers during the touching time.

- ➢ touchesEnded:withEvent:—end the touch process, where the finger or fingers are taken off the screen. It provides an appropriate time to clean up any work which was handled during the movement sequence.

- ➢ touchesCancelled:WithEvent:—be invoked when framework must respond to a system interruption of the current touch event.

Multi-touch in iPad

By setting the GUI property multipleTouchEnabled to YES when building or setting boolean function isMultipleTouchEnable to YES when writing code, the application of iPad will

enable its interaction with multi-touch events. iPad will respond more than one touch at one time. When multi-touch is set enabled, the touch events will return a callback of a set of touches.

Different number of touches at one time and the following movement of each touch until touches left the touch screen will form a variety of gestures. No matter how long these steps will take, it must be one gesture as long as one of touches is still on the touch screen. One of the most important gestures in iPad is the pinches, which is defined by that two finger touches in a single touch event and the two finger touches must move together or a part in a single movement. Pinches are widely used in many iPad applications. For example, zoom in and zoom out in safari, mail, and photo library.

How to detect a pinch gesture? The process of detecting a pinch gesture is as follow. First, when a touch event begins, there exist two touch points because pinch is a gesture based on two touches. Second, if two touch points exists, the initial Euclidean distance will be recorded. Third, when two touch points move, Euclidean distance is detected and recorded during all the moving time. If the new Euclidean distance is more than the initial Euclidean distance and the absolute difference between them excesses a threshold (which can be adjusted), we will take this gesture as a pinch with zoom in purpose. And if the new Euclidean distance is less than the initial Euclidean distance and the absolute difference between them excesses a threshold which can be adjusted, we will consider this gesture as a pinch with zoom out purpose.

CHAPTER 3

MOBILE BIOIMAGE INFORMATICS

The iPad for bioimage informatics tool will be divided into two parts, function one and function two. This chapter mainly focuses on the function one that is mobile bioimage informatics function.

Bioimage Segmentation

In the computer vision field, segmentation is considered as an important but difficult problem. Over decades, researchers conducted abundant intensive researches, but still are not able to provide fully automatically optimal solution for many discretional images. Researchers realize and reach an agreement that with the valuable information human inputted, the segmentation results would be more accurate and easy to achieve. Thus, interactive image segmentation develops based on that agreement, and there have been several important and mature algorithms, such as intelligent scissors [38], Bayes matting [39], graph cuts [40], and GrabCut [41].

Biomedical image segmentation plays an important role in clinic and research. Many biomedical fields need the help from image segmentation, for example, quantification of tissue volumes [42], diagnosis [43], localization of pathology [44], study of anatomical structure [45], treatment planning [46], partial volume correction of functional imaging data [47], and computer

integrated surgery [48], [49]. Interactive biomedical image segmentation is widely used. With the

help of image segmentation algorithms, researchers usually use computer and its accessories, such

as keyboard, stylus, mouse and paint board, to directly input the information which usually is the

delineation of anatomical structures and other regions of interest, and specify what the

foreground/background is and what part they really care about. Interactive biomedical image

segmentation brings the normal and diseased anatomy for medical clinics and research and plays

an important role in diagnosis and treatment planning [50].

What is GrabCut

GrabCut algorithm is based on the paper [40] which finished the segmentation on a

monochrome image. The grey scale image is represented to a grey value array $Z = (z_1, ... z_n, ... z_N)$.

The "opacity" value is represented to $\underline{\alpha} = (\alpha_1, ... \alpha_N)$ at each pixel. The value of α_n should be

between 0 and 1, but for hard segmentation its value is either 0 for background or 1 for

foreground. And parameter $\underline{\theta}$ represents background and foreground grey-level distribution. An

energy function $E(\underline{\alpha}, \underline{\theta}, Z) = U(\underline{\alpha}, \underline{\theta}, Z) + V(\underline{\alpha}, Z)$, where term U evaluates the fits of opacity

distribution and term V is a smooth term, is designed for the purpose such that when it is

minimum there would be a good segmentation result. After energy function is fully defined,

graph cut will use a standard minimum cut algorithm $\hat{\underline{\alpha}} = \arg\min_{\underline{\alpha}} E(\underline{\alpha}, \underline{\theta})$ to perform hard

segmentation [40], [51].

GrabCut, compared with graph cut, improves in three aspects. Firstly, images used for segmentation expend to color space image by applying the Gaussian Mixture Model (GMM). Secondly, a more powerful iterative algorithm is used to generate a more adaptive parameter learning and estimation procedure, comparing with one-shot minimum cut estimation algorithm. Thirdly, it reduces the user interactive work by using incomplete labeling that user only indicate the background for trimap which is a user-generated mask map made up of black, white, and grey three tones.

For color space modeling, GrabCut use Gaussian Mixture Model and follow a practice of paper [52], [39]. It introduces an additional vector $K = (k_1, ... k_n, ... k_N)$ to each pixel as a GMM component, so that the energy function $E(\underline{\alpha}, \underline{\theta}, Z) = U(\underline{\alpha}, \underline{\theta}, Z) + V(\underline{\alpha}, Z)$ have changed to $E(\underline{\alpha}, k, \underline{\theta}, Z) = U(\underline{\alpha}, k, \underline{\theta}, Z) + V(\underline{\alpha}, Z)$ where term U is still an opacity distribution term but with taking account of color GMM models and term V is a smooth term with slightly changes to adapt color spaces.

GrabCut uses an iterative energy minimization method. It benefits those refinements of opacities $\underline{\alpha}$ and the color GMM component parameter $\underline{\theta}$ work fully automatically. The whole iterative procedure consists of following steps. After assigning GMM component to each pixel, program learns GMM parameters from data Z in order to estimate segmentation by using min cut to energy function $E(\underline{\alpha}, k, \underline{\theta}, Z)$. These steps repeat until energy function $E(\underline{\alpha}, k, \underline{\theta}, Z)$ converge it means energy function $E(\underline{\alpha}, k, \underline{\theta}, Z)$ ceases to decrease significantly.

The idea of incomplete trimap labeling is that with user's indication of background (no hard foreground labeling needed) iterative algorithm takes background pixel as firm pixel and deals other pixel as provisionally retracted foreground pixel to execute minimization.

The following Figure 1 show the performance of GrabCut on natural images.

Figure 1 GrabCut results in natural images. Figure courtesy: [41].

Interactive bioimage informatics on iPad

Because iPad can be connected to the Internet anytime and anywhere, the prominent features of its portability and ubiquitous Internet connection for mobile bioimage informatics applications are self-evident. When applying iPad as a mobile bioimage informatics client, the user interaction can be performed on the portable iPad, while the time-consuming procedures e.g., iterative graph-cut optimization for image segmentation [41], can be performed in a much more powerful back-end server, e.g., supercomputers or clusters. Importantly, the communication between iPad and back-end server is through the ubiquitous 3G or Wi-Fi connection channel. The portability and ubiquitous connection of iPad makes it a very attractive platform for mobile bioimage informatics applications. The concept here is akin to that in the Google Goggles application [53] which seamlessly integrates image management on smartphones and content-based image search and management on the back-end server or cloud.

In this section, we present a prototype mobile bioimage informatics system that performs user interaction on iPad and conducts real bioimage computing at the server side. The prototype system is a client/servlet model based on 3G wireless network. This back-end server performs real image segmentation that is based on the "Grab-cut" algorithm designed in [41]. The user only needs to tap two points on the iPad screen, and sends the point information to the remote servelet and download the segmentation result after servelet finishes the computing. The prototype system we designed and implemented leverages faster image processing speed at the server, because iPad

is not good at large-scale computing but is good at fast network connection and with friendly user interface.

In our prototype system, as illustrated in Figure 2, user loads image to GUI, taps two points (e.g., yellow dots in Figure 3) on iPad, send the points information to a server, and receives the segmentation result from the server. Specifically, at the iPad side, user just moves fingers and tap on the screen twice to select two input points (Figure 3), which are the top left and bottom right corners of a rectangle covering the foreground. This rectangle information along with the image itself is sent to the server via built-in Internet communication channel. On the server side, an Apache tomcat web server and a Java application program handle parameter interpretation and image transfer. The Java application also communicates with the graph-cut based image segmentation module [41] to receive and send the image segmentation parameters and results.

Structure and procedures

This prototype system involves UITouchPhaseBegan and UITouchPhaseEnded two life cycles. When user touches the screen, it triggers the UITouchPhaseBegan and when use's finger leaves the screen, the system will record the point coordinate information.

For the client part, it is the iPad device. According to the accurate built-in multi-touch function provided on iPad, Users just move their fingers and tap on the screen twice for two points to input segmentation information, in here we usually tap one point at the upper left and another point at bottom right to come into being a rectangle. The foreground is usually inside the

rectangle. Our app collects the two points information and sends them to the remote servlet formatting in http request through ASIHTTPRequest iOS package [54]. When the remote processing finished, users would be able to download the segmentation result.

For the servlet part, it is apache tomcat web server. A file upload function is added to original tomcat web server, which is provided in [55], in order to listen and handle http post requests from iPad. With http requests, it writes a txt file and an image file to web server upload folder. A Java application using eclipse is designed to retrieve segmentation parameters from txt file and also image file in web server upload folder. When the Java application runs, it scan web server upload folder in every minute, if it finds the txt file and image file, it retrieve the points values and image value and invoke MATLAB application to calculate by passing the segmentation parameters to the MATLAB jar package which is built within local MATLAB. The MATLAB application we used here is Interactive Segmentation Tool-box, which is an implementation of 'Lazy Snapping' and 'GrabCut': Based on Interactive Graph Cuts. This MATLAB tool box was implemented by Mohit Gupta and Krishnan Ramnath who were graduate students of Carnegie Mellon University.

After finishing computing, MATLAB application will show the result and Java application will send the result image to download folder which is in apache tomcat web server. We need to observe the whole process and when the MATLAB shows the result, we will tap the download button on iPad then iPad would send http get request to web server download folder and the result image will be displayed on iPad.

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iPad Client

```
┌──────────┐
│  Start   │
└────┬─────┘
     │        Tap Select Image button
     ▼
┌──────────────┐
│ Select image │
└──────┬───────┘
       │
       ▼
┌──────────────┐
│ Display image│
└──────┬───────┘
       │        Tap Start Touch button
       ▼
┌──────────────┐
│ Tap two points│
└──────┬───────┘
       │        Tap Upload to Process button
       ▼
┌──────────────┐
│Upload to server│
└──────┬───────┘
       │        Wait and then tap Display Result button
       ▼
┌──────────────┐
│ Download  and│
│ display result│
└──────┬───────┘
       │
       ▼
┌──────────┐
│   End    │
└──────────┘
```

Java console

```
        ┌──────────┐
        │  Start   │
        └────┬─────┘
             │
             ▼
        ╱──────────╲          No file
       ╱   Scan      ╲  ──────────┐
      ╱  WebServer    ╲
      ╲ upload folder ╱
       ╲             ╱
        ╲───────────╱
             │   Files received
             ▼
      ┌──────────────┐
      │ Call MATLAB  │
      │ to execute   │
      └──────┬───────┘
             │
             ▼
      ┌──────────────┐
      │ Put result to│
      │  WebServer   │
      │download folder│
      └──────────────┘
```
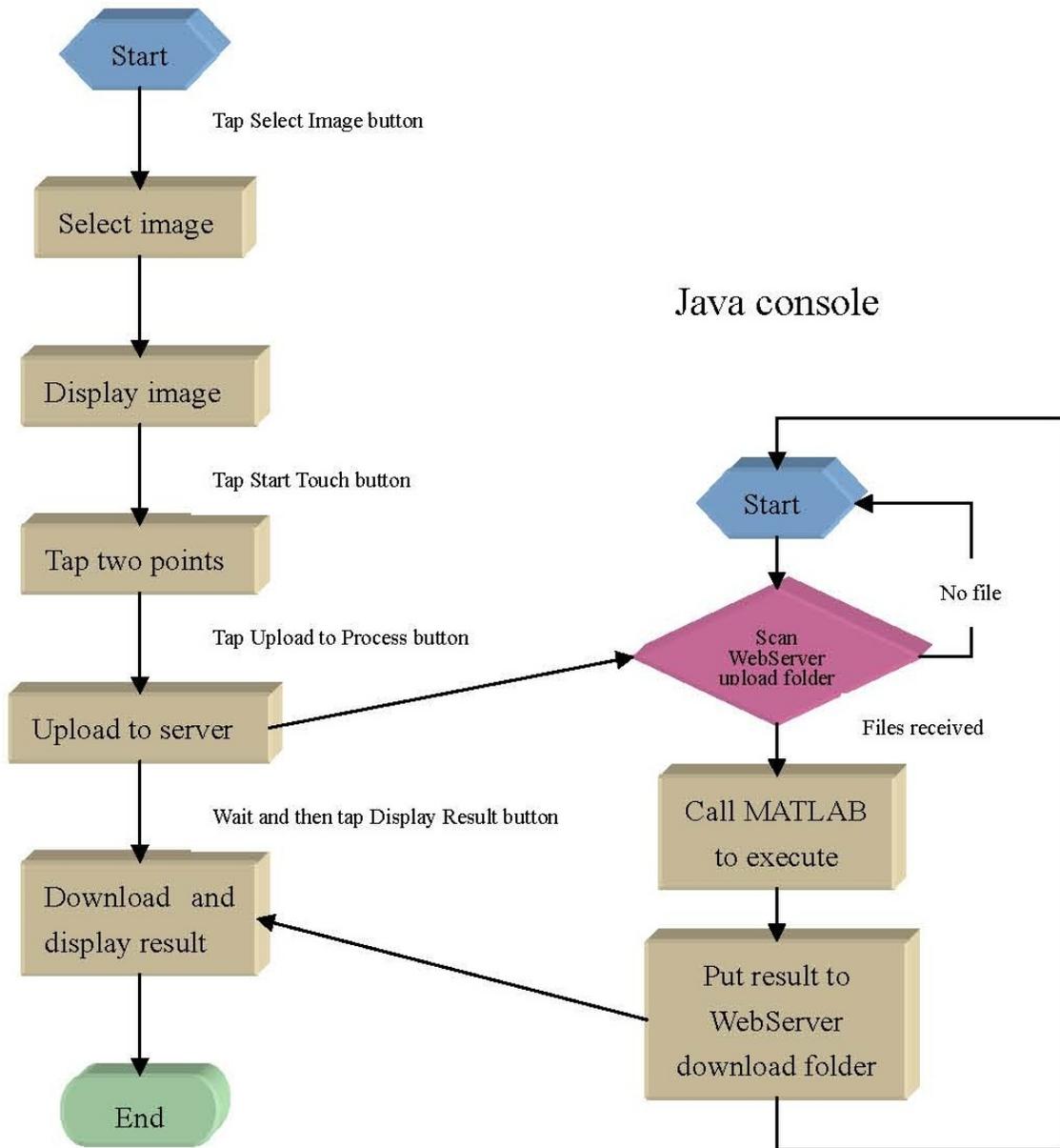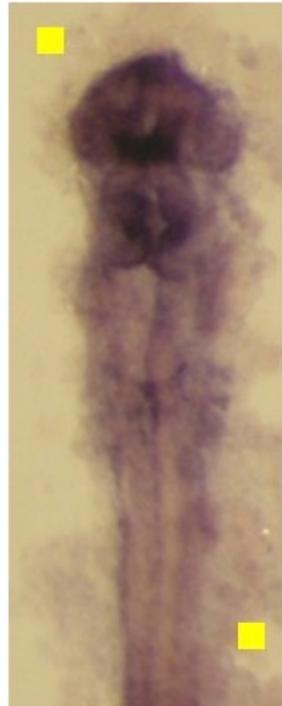
Figure 2 Flowchart of mobile bioimage informatics

Figure 3 A screenshot of using the iPad interface for mobile image segmentation. The two yellow dots were selected for graph-cut based semi-automatic segmentation [41].

Performance of mobile bioimage informatics

Because iPad can connect to Internet anytime and anywhere, the prominent features of its portability and ubiquitous connection for mobile interactive image segmentation are self-evident. Since the graph-cut based image segmentation algorithm has already been evaluated in [41], here we only show a few exemplar cases of using our prototype system for image segmentation. The results in Figure 4, Figure 5show that the segmentation results are reasonable, although the segmentation result can be further fine-tuned by interactive labeling on iPad. The communication between iPad and the server is very fast (within a few seconds).
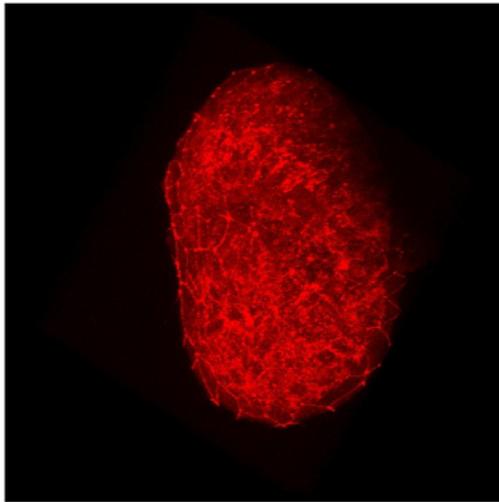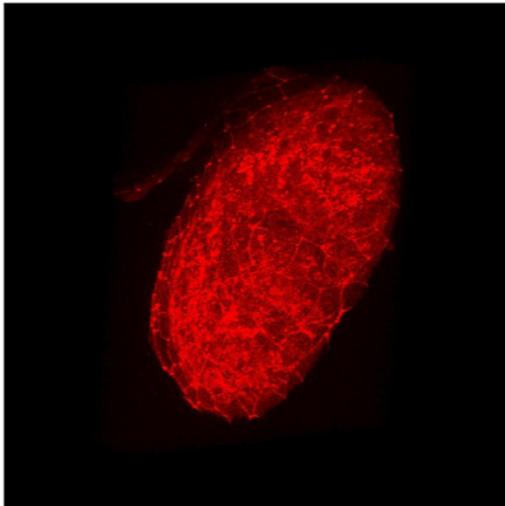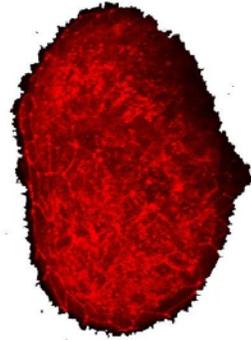
19

(a)

(b)

Figure 4 Example one of mobile bioimage informatics. (a)-(b): two examples of segmentation results of zebrafish microscopy images. The left and right images are original and segmented images respectively in (a) and (b). The two tap points used for iterative graph-cut segmentation are shown by the yellow dots. Figure courtesy of Dr. Weiming Xia.
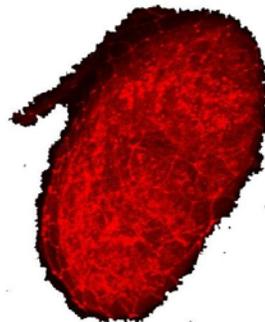
(a)

(b)

Figure 5 Example two of mobile bioimage informatics. (a)-(b): Examples of biomedical cell image segmentation results. The left and right images are original and segmented images respectively. Figure courtesy: Broad Institute.

It should be noted that although the prototype system presented here is still very preliminary, it demonstrated the possibility and feasibility of integrating front-end user-friendly iPad client and powerful server for bioimage informatics applications. In the future, many more bioimage computing services can be installed in the server side to facilitate the promising paradigm of using iPad for mobile bioimage informatics.

CHAPTER 4

BIOLOGICAL OBJECT TRACING AND SEGMENTATION

The iPad for bioimage informatics tool will be divided into two parts, function one and function two. This chapter mainly focuses on the function two that is a biological object tracing function.

Structure of tracing function

We developed a general iPad prototype system that allows users to draw the contours of biological objects. Figure 6 show the operation flowchart. When a user draws a contour at the edge of the region of interest in an image, it will show the contour curves at the same time. Also, users can save the contour image for the following step of segmentation. This prototype system provides a faster way to draw a biological object tracing curve, in comparison to the typical way of using mouse on desktop/laptop computer. Moreover, iPad avoids the uncomfortable feeling of clicking and moving mouse, permitting them to accurately describe the image foreground regions using a what-you-see-is-what-you-get graphical interface that maps the contour immediately and automatically on top of the bioimage on the screen.

In many biomedical image segmentation applications, manual tracing of image objects is widely used to provide either benchmark data or training samples [28, 32]. This prototype system was designed and implemented to showcase that iPad can be used for interactive tracing of

biomedical image objects. Specifically, when a user moves his/her finger to draw a contour at the object boundary, our prototype system GUI will save and visualize the contours on the image simultaneously, as illustrated in Figure 7. In comparison to traditional manual tracing using mouse on desktop computers, iPad GUI provides more natural and user-friendly human-computer interaction, faster tracing, and more accurate results (comparison results shown in following). In addition, the iPad multi-touch technology can facilitate zooming in or out the image in order to have an adaptive view of the object (Figure 7).

This prototype allows user to draw a contour for a foreground with one finger. When user moves the finger on iPad's screen, iPad's multi-touch screen will precisely record every movement of finger and the program will responsively store every point coordinates to a stack first in memory and then to flash disk. iPad will display a red curve on screen which is drawn by the program to connect the previous point with current point. With the program continuing draw the red curve, a red contour will display above the original image when user stops move the finger.

This prototype system involves four phase, UITouchPhaseBegan, UITouchPhaseMoved, UITouchPhaseStationary, and UITouchPhaseEnded. When a user touches the screen and the finger does not leave away from the screen, it triggers the UITouchPhaseBegan; and then user began to move the finger on the screen surface, it triggers UITouchPhaseMoved. While moving the finger, our system records the previous touch point and current touch point, after computing the Euclidean distance between these two points, and if the distance greater than 1, the system will record current point coordinate information and put data into a stack and draw a line

between these two points. iPad will display a red line on screen which is drawn by program to connect the previous point with current point. We are able to control the accuracy of recording moving point.
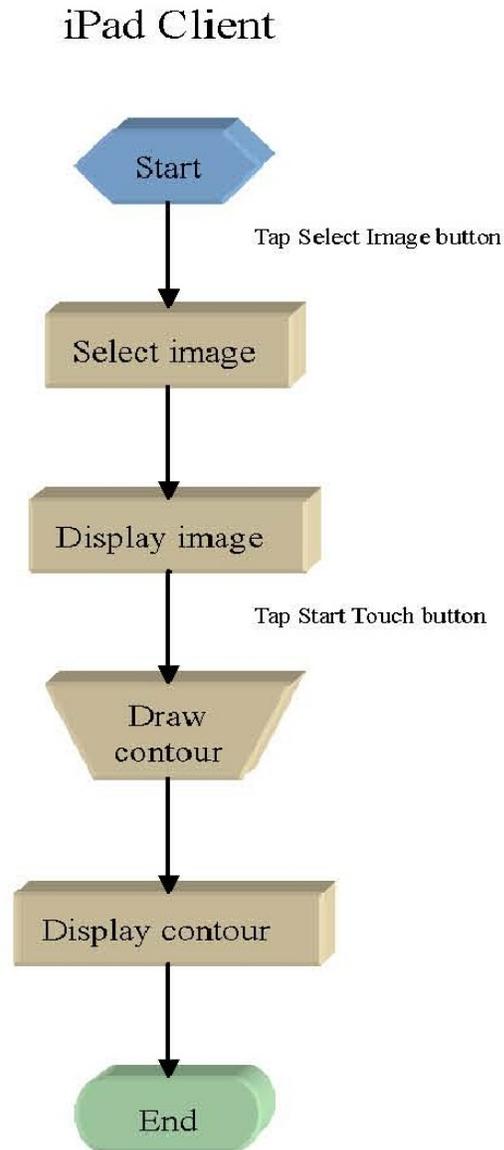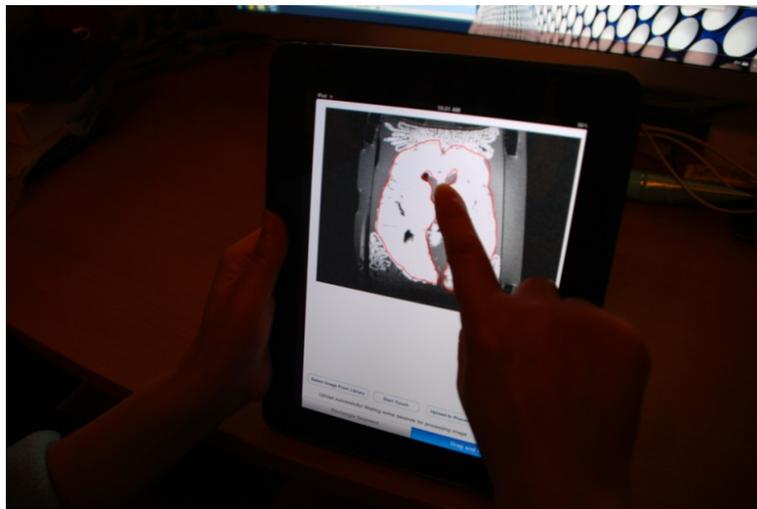
iPad Client

Figure 6 Flowchart of biological object tracing

(a)

(b)

Figure 7 Biological object tracing GUI. (a)-(b): Two screenshots of using the iPad GUI for interactive brain boundary tracing. The left figure (a) shows the zoomed-out image. The right figure (b) shows drawing brain boundary after the image is zoomed in.

In particular, our system is set to record every movement of a new point. The system will take the current point as the previous point if a new movement occurs. If the user stops moving the finger but still put the finger on the screen surface, UITouchPhaseStationary will be triggered and system will be noticed that the whole movement is paused but over. When user pulls the finger away, UITouchPhaseEnded will be triggered and the movement is over and all the contour points coordinate information are all recorded into the stack. With the user continuing moving the finger and program continuing draw the red line, a red contour will display above the original image when user stops move the finger.

In the following sections, this prototype system will be used for four applications of brain MRI image tracing, neurite tracing, zebrafish segmentation, cell segmentation, and somite annotation.

Brain MRI image tracing

We applied the iPad interactive tracing system on brain MRI images. The subjects' brains were donated from a centenarian study. The MRI dataset was acquired post-mortem on a 3T GE MRI scanner. As a result, the post-mortem preparation caused cluttered background in MRI scan (Figure 8). Therefore, interactive manual tracing of the brain from cluttered background is warranted to achieve satisfactory segmentation accuracy. Similarly, the iPad and mouse based boundary tracing methods mentioned above were applied to delineate the brain boundaries, and Figure 8 shows five examples.
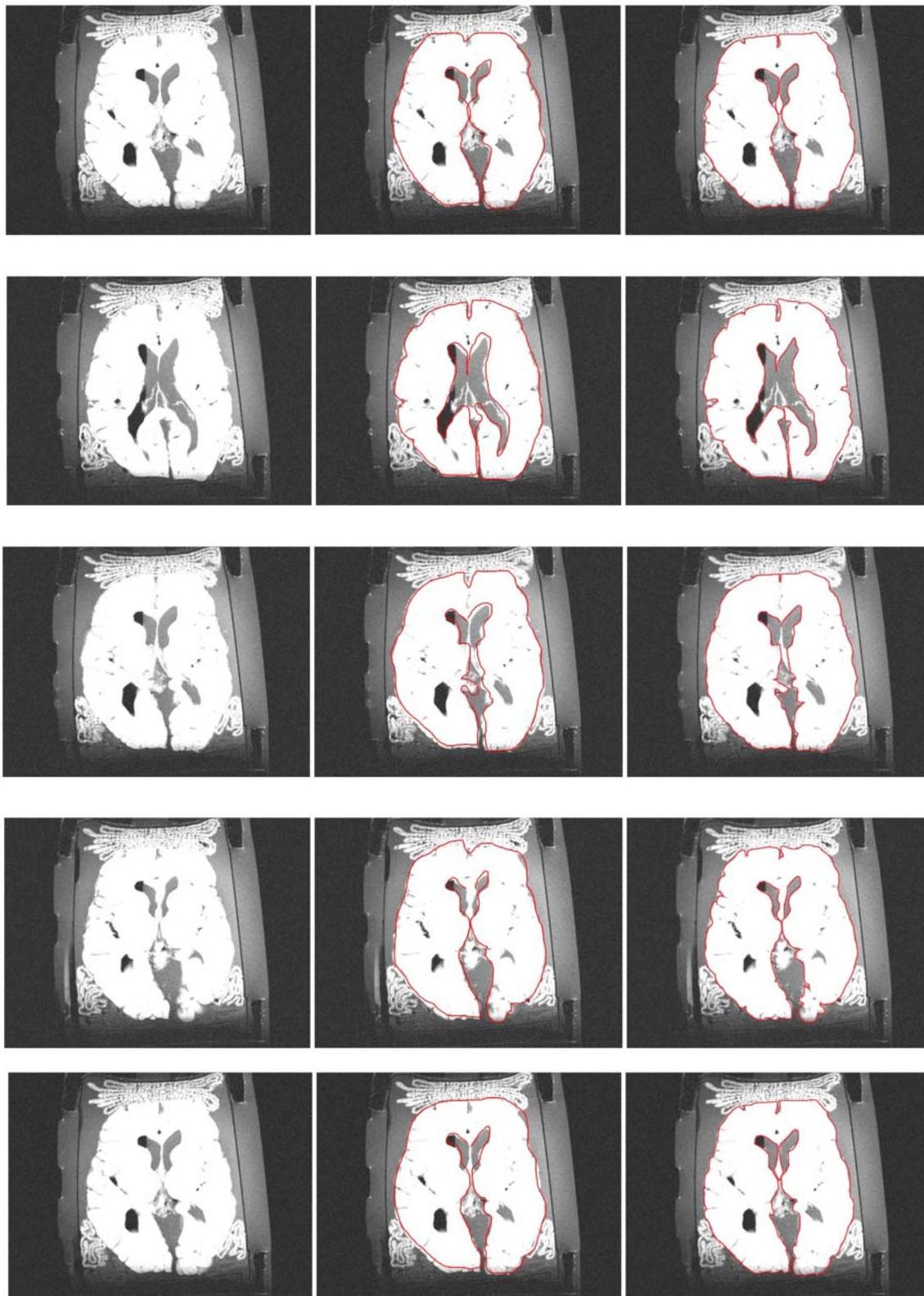
Figure 8 Comparison of brain MRI image tracing by using iPad and mouse. Original image(left), iPad draw image(middle) and mouse draw image(right). Figure courtesy: Dr. L Stephen Miller.

Table 1 and Figure 9 show the time cost for the drawing, the unit of time cost is second.

Table 1 Time comparisons of brain MRI image tracing

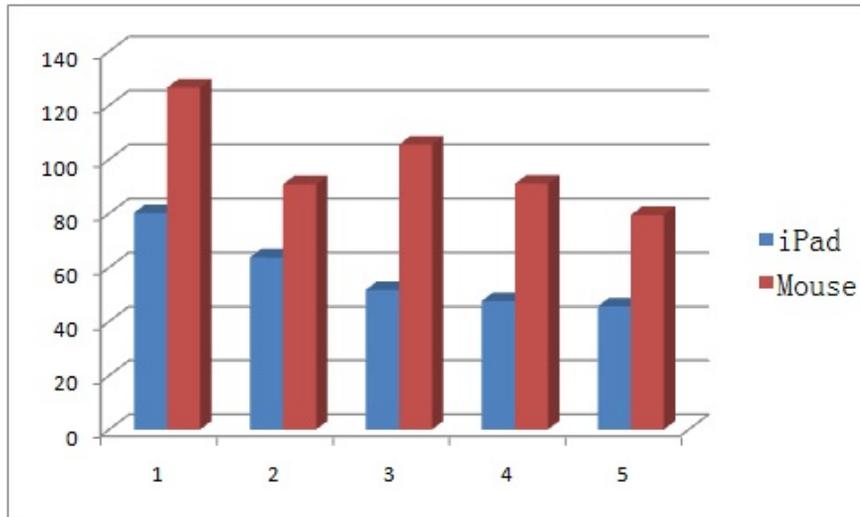| iPad (second)	mouse (second)
80.2	126.6
63.7	90.8
51.7	105.5
47.6	91.0
45.6	79.4

Figure 9 Time comparisons of brain MRI image tracing

It is evidently true that both iPad and mouse tracing methods achieved satisfactory boundaries. However, to achieve comparable segmentation results, the mouse based tracing method took 40% more time than the iPad based segmentation. Figure 9 shows the time costs for segmentations of five different brain images using both methods respectively. It can be seen that the iPad based segmentation is consistently faster than mouse based segmentation. In addition, users feel more comfortable and natural in using iPad for interactive boundary tracing. The segmented brains from MRI images can then be used for other quantitative analysis such as area or volume measurement in the future.

Neurite tracing

Modern fluorescence microscopy technology has made high-content screening of neuronal assays an important approach to understanding molecular pathways or identifying potential new therapeutic treatments. Morphological quantification of fluorescence microscopy images plays an increasingly important role in high-content neuronal screens. In these applications, typically, neurite tracing is a prerequisite step in high-content screening of neuronal assays [56].

In this experiment, we applied the iPad prototype system on neurite images to evaluate its performance on neuronal tracing. The testing images were obtained from a neuron screening in [57]. As shown in Figure 10, we randomly choose five images as examples, and their complexities of neuronal cell networks vary from lower to higher. For the purpose of comparison, the same set of neurite images were traced via using the mouse on desktop computer. Then the time spent for those tracing tasks were recorded.
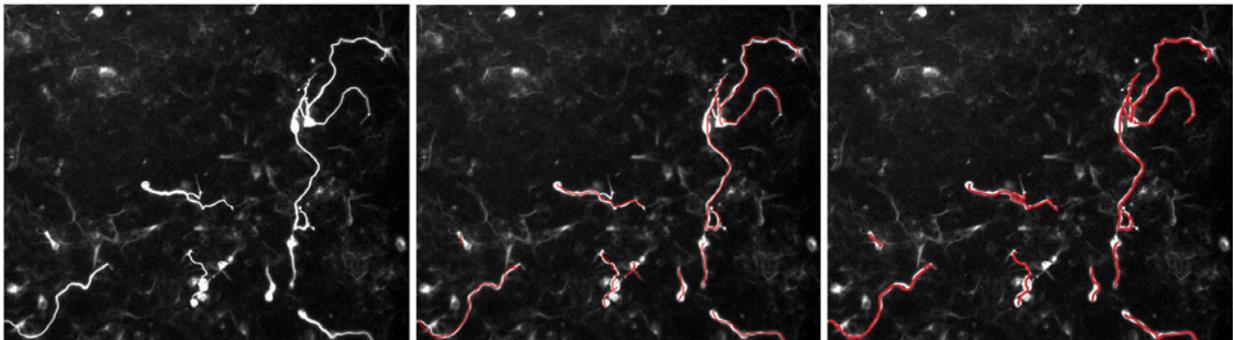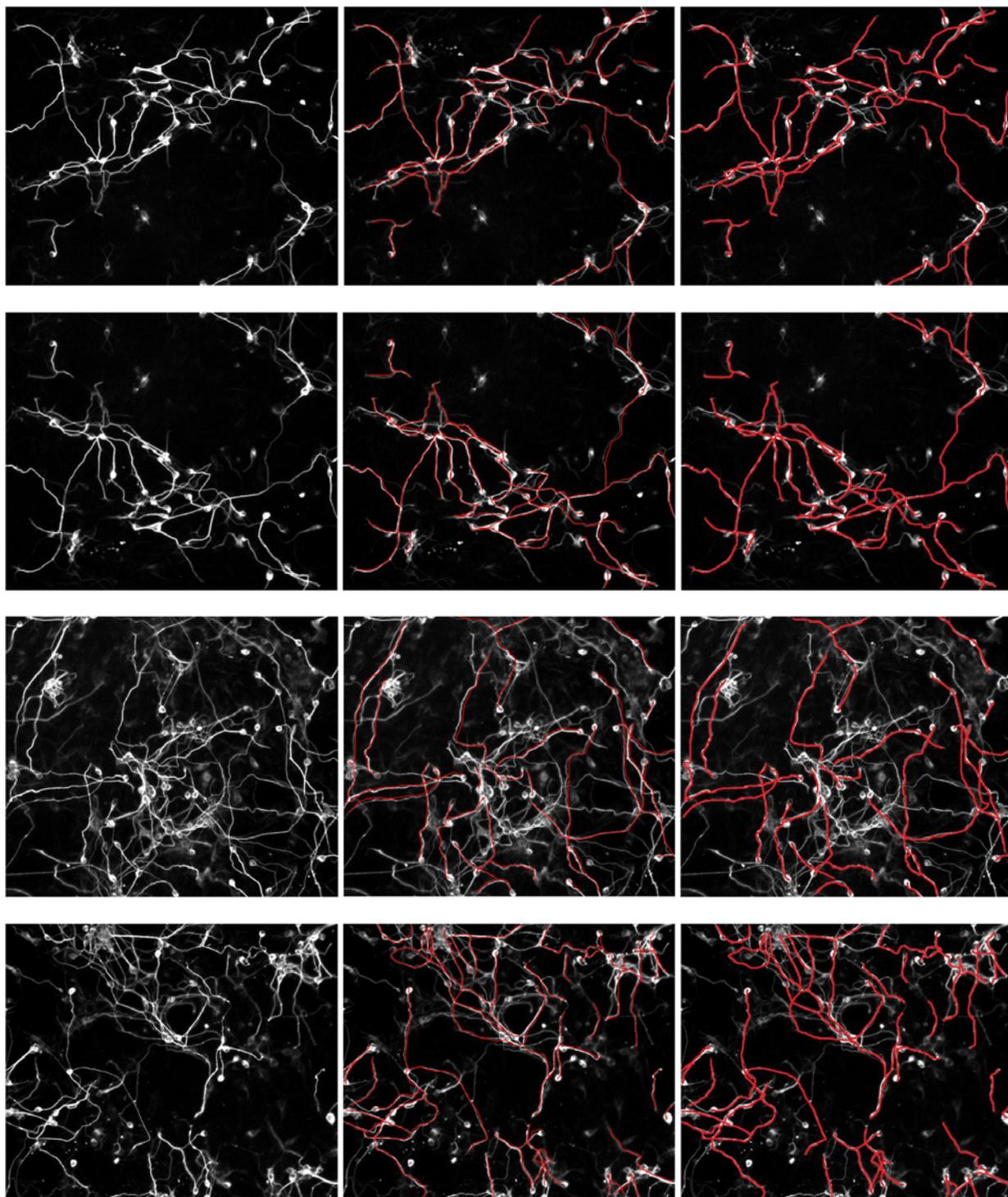
Figure 10 Comparison of neurite tracing by using iPad and mouse. Five examples (from top to bottom panels) are shown here: original image (left), iPad tracing result (middle) and mouse tracing result (right). Figure courtesy: [57].

Table 2 and Figure 11 show the time cost for the drawing, the unit of time cost is second.

Table 2 Time comparisons of neurite tracing

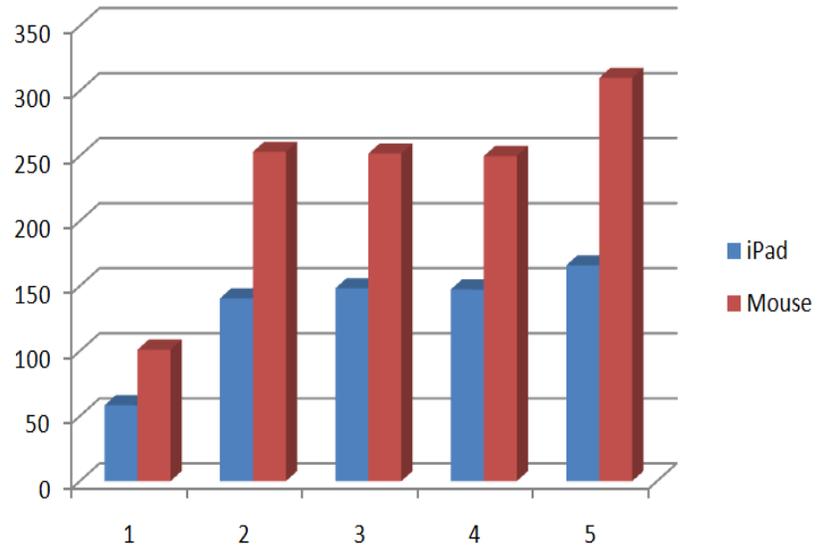| iPad (second)	mouse (second)
58.3	101.1
140.5	253.2
148.3	251.9
147.4	249..9
165.9	310.0

Figure 11 Time comparisons of neurite tracing

After quantifying the comparisons in Figure 11, we found that iPad will save significant amount of time, in comparison with mouse tracing. In general, at least 40% time will be saved, and in the certain cases, almost 50% time will be saved. It is interesting that the more complex the neurite image is, the more time is likely to be saved by using iPad. This result suggests that iPad-based neurite tracing is much faster than traditional mouse tracing on desktop computer, while achieving the same tracing quality.

Zebrafish segmentation

Zebrafish has recently emerged as an invaluable vertebrate system for disease modeling and drug discovery [58]. During the past two decades, zebrafish has been demonstrated to be a suitable vertebrate organism for both forward and reverse genetic screening [58, 59]. One of the major advantages of using zebrafish as animal model is that hundreds of transparent embryos allow easy manipulation and examination of zebrafish developmental processes, because all blastomere divisions, gastrulation movements, as well as the major events of morphogenesis and organogenesis occur within 24 hours. Besides time-lapse image acquisition for developmental alterations in living embryos, in situ hybridization and immunohistochemical staining have revealed zebrafish mutants. In addition, by fusing green fluorescent protein (GFP) to genes or promoters of interest, it is possible to follow the activity of a target gene/promoter in living zebrafish. Recently, computerized analysis and management of zebrafish bioimages has emerged as a research topic [60].

In this experiment, we applied the iPad prototype system on zebrafish microscopy image datasets [60, 61]. We traced the boundaries of zebrafish in ten microscopy images, and ten examples are shown in Figure 12. Similarly, for purpose of comparison, we performed boundary tracing on these zebrafish images using traditional mouse tracing on a desktop computer.
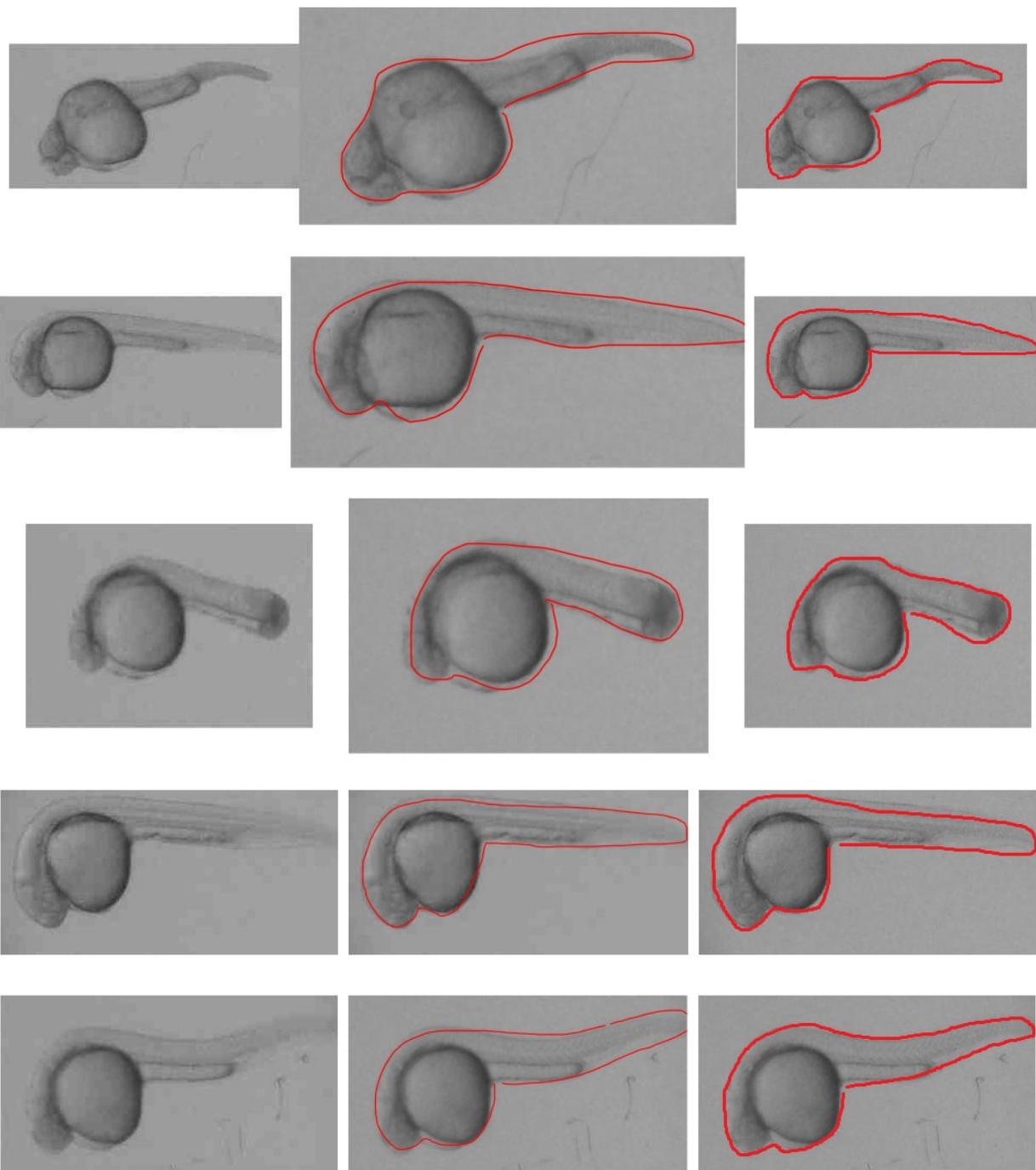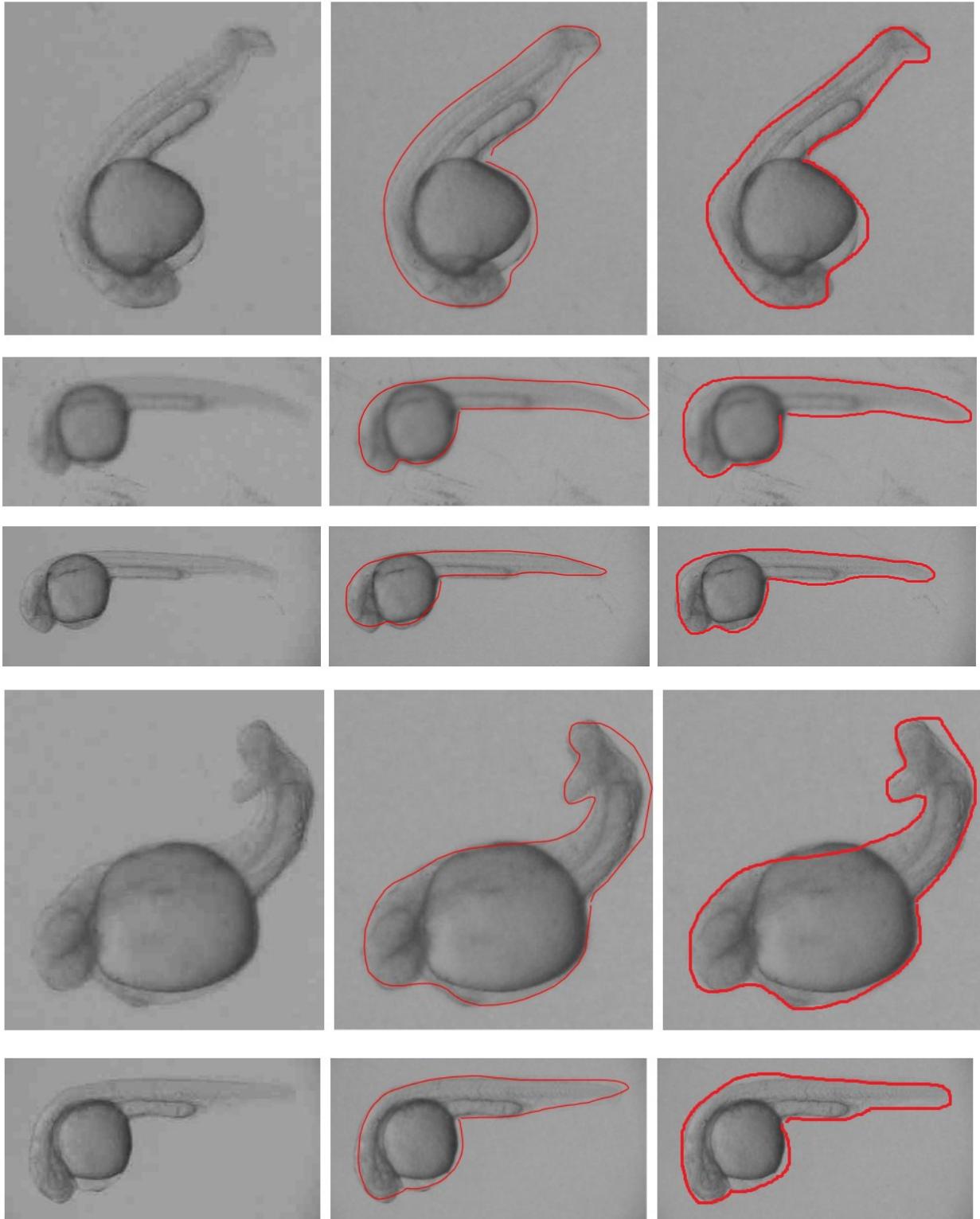
Figure 12 Comparison of zebrafish segmentation by using iPad and mouse. Original image (left), iPad draw image (middle) and mouse draw image (right). Figure courtesy: Dr. Weiming Xia.

By visual inspection, the iPad based segmentation is even smoother that that obtained by mouse segmentation. Also, We recorded the time (in seconds) used to draw satisfactory zebrafish boundary contours using iPad and mouse respectively, and reported the time differences for ten cases of zebrafish images in Figure 13. It is evident that the iPad based segmentation consistently took much less time to accomplish the boundary tracing. On average, the iPad based segmentation took 30% time less than the mouse based segmentation. In addition, we compared the accuracies of these two set of segmentations based on benchmark datasets provided by two experts showing in Table 3.

Table 3 Time and accuracy comparision of zebrafish segmentation

| iPad effective pixel	mouse effective pixel	manual effective pixel	iPad&mouse overlap pixel	iPad&manual overlap pixel	mouse&manual overlap pixel	time_iPad (second)	time_mouse (second)
15399	16822	15348	15051	14689	15194	4.7	6.1
14490	14272	14095	13572	13437	13509	3.7	5.4
16960	16115	16711	15146	15537	14803	4.3	5.4
17834	15883	16736	15476	16009	15425	5.8	6.2
30482	24993	28141	24812	27770	24436	4.7	7.5
19465	19922	19017	17951	17750	17690	4.2	5.8
14151	15158	14828	13230	13573	14092	4.6	5.9
39450	37038	38064	36394	37138	35869	4.6	9
14563	15112	13947	13963	13663	13560	4.1	6
13692	12603	13202	12117	12470	12033	4.5	6.4
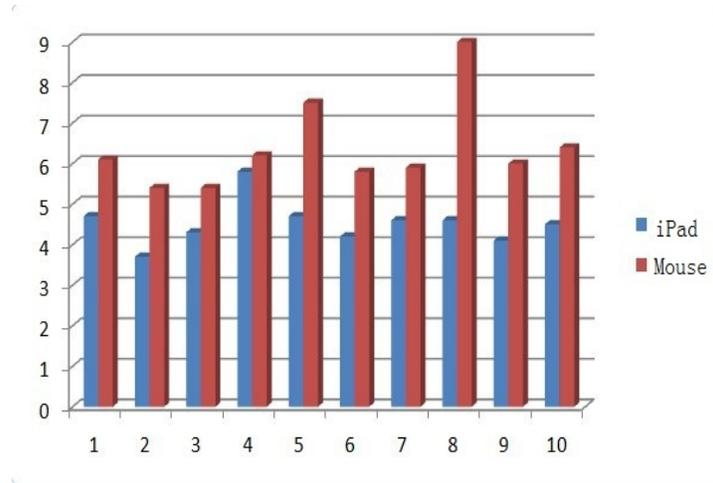
Figure 13 Time cost comparisons of zebrafish segmentation

For analysis data purpose, as the Table 3 and Figure 14 showing that, we assume the manual segmentation result is the best result, and we try to find the maximum overlap area (area 5), which the iPad segmentation and mouse segmentation cover the manual segmentation separately, with the lowest self area cost (area 1, 3, 2, 4). The area overlap was used as the metric for comparison. It means that we need calculate the percentage that the over lapped area takes from the total area, and the higher this percentage is, the more accurate it will be. In the end we use this percentage divided by the time to obtain the efficiency term.
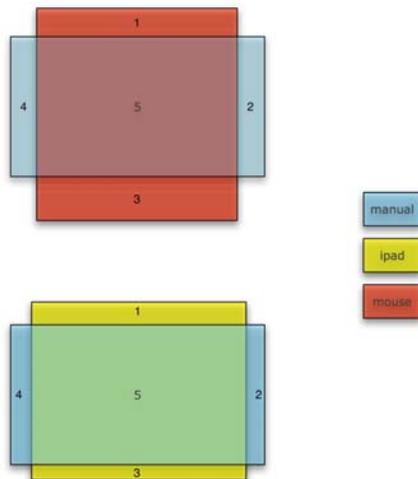

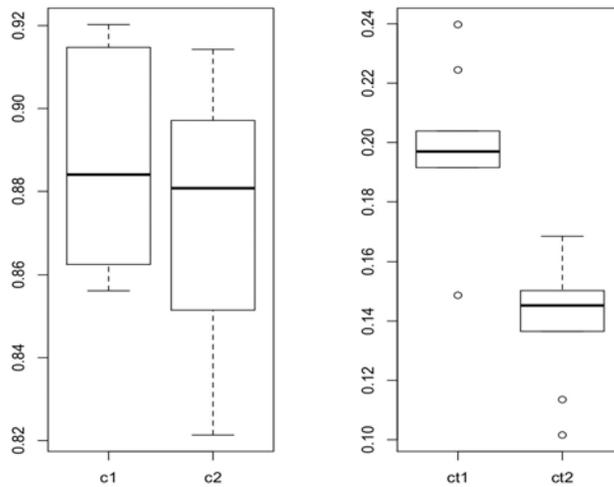Figure 14 Analysis method of zebrafish segmentation

We obtain the following formula

$$c = \frac{A \cap B}{A + B - A \cap B}$$

$$ct = \frac{c}{t}$$

And then we use R as the computing tool, and input the command as following:

data=read.table("ipad.txt",header=T)

area1=data[,1]+data[,3]-data[,5]

area2=data[,2]+data[,3]-data[,6]

c1=data[,5]/area1

c2=data[,6]/area2

c=cbind(c1,c2)

ct1=c1/data[,7]

ct2=c2/data[,8]

ct=cbind(ct1,ct2)

par(mfrow=c(1,2))

boxplot(c)

boxplot(ct)

And the plot result will be generated as Figure 15. In Figure 15, c1 and ct1 stands for iPad, and c2 and ct2 stands for mouse. After analyzing the plot result, we will reach the conclusion that It is evident that the iPad based segmentation has higher overlap and less variance across different zebrafish images than the mouse based segmentation. Hence, the evaluation results demonstrate that iPad based zebrafish segmentation has better accuracy, saves significant amount of time, and is more user friendly. In the future, these segmented zebrafish images can be used for morphological analysis such as shape and area measurements [60, 61].

(a)   (b)

Figure 15 Analysis result of zebrafish segmentation. (a) Accuracy comparison. (b) Accuracy comparison with time effect. c1 and ct1 stand for iPad, c2 and ct2 stand for mouse.

Cell segmentation

Accurate cell segmentation from microscopic images has been an active research topic in many biological studies, since it is required for subsequent comparison or classification of cell morphology and behavior. In recent years, there has been significant amount of research work on the development and validation of automated methods for cell image segmentation [28, 62]. In spite of active research and significant progress in the literature, fully automated and robust segmentation of cell images is still an open and challenging problem, especially when dealing with significant inherent cell shape and size variations in image data and dealing with touching cells. Examples include cases in which the intensity contrast between cell and the background is low, in which there are significant differences in shapes and sizes of cells, and in which we are

dealing with images of low quality. As a result, manual cell segmentation is still warranted in many biological imaging applications. In addition, manual cell segmentation can provide benchmark data for comparisons and evaluations of cell segmentation algorithms and software systems.

In this experiment, we applied the iPad prototype system for cell segmentation from fluorescence microscopy images [63] (Figure 16a). Figure 16b shows an example of typical segmentation results. It is evident that the segmentation boundary is reasonably accurate. Again, the iPad-based segmentation is much faster than using mouse on desktop computer. It takes only half of the time that is used by using mouse on desktop computer. Once the cells are segmented, they can be used for the following work of cell pattern classification [64].
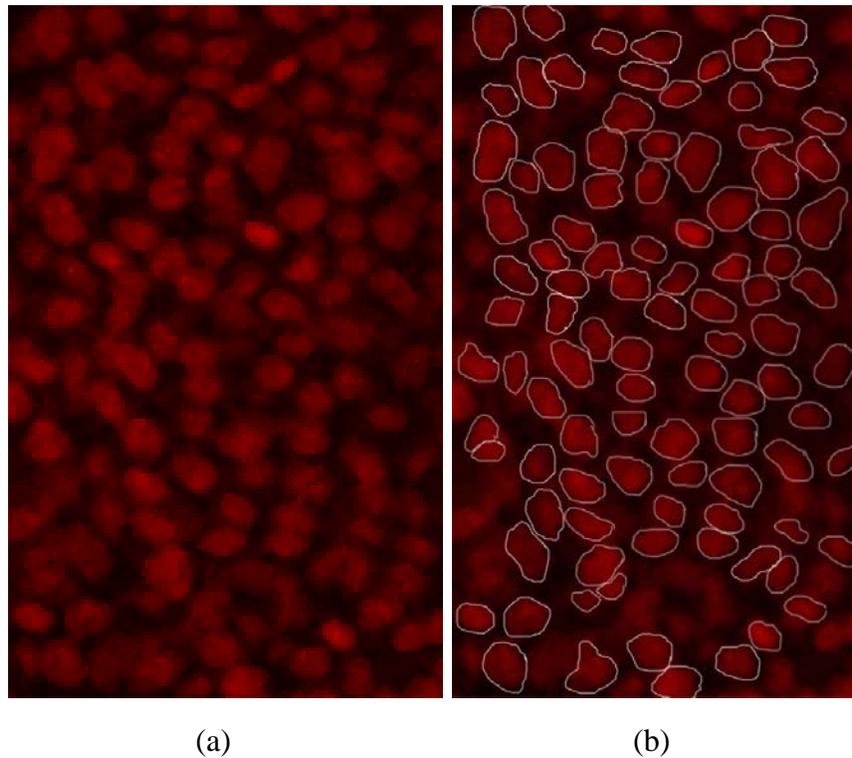

(a)   (b)

Figure 16 Example of cell image segmentation. Cell image (a) and its segmentation results (b). Figure courtesy: Dr. Scott Holley.

Somite annotation

Somitogenesis is the process by which the segmented precursors to the vertebral column and musculature are generated during vertebrate embryogenesis [65]. Morphological segmentation occurs when cells within the anterior of the mesenchymal presomitic mesoderm epithelialize to form bilateral pairs of somites. This process is reiterated in an anterior to posterior direction, adding new somites as the embryo grows at its posterior. Morphological segmentation is governed by the somite clock, which creates oscillations in gene expression-predominantly of genes in the Notch pathway-within the mesenchymal presomitic mesoderm [65]. These oscillations manifest themselves as repeated cycles of activation and repression of transcription, thus creating stripes of gene expression that traverse the presomitic mesoderm in a posterior to anterior direction. Zebrafish has been used as a modeling system to study somitogenesis and more generally to study how the sum of the function of many individual genes gives rise to higher levels of organization such as the dynamic yet stable cell behavior inherent in multicellular patterns/structures [65]. Zebrafish embryos are transparent and thus are particularly well suited for microscopic imaging and embryological experiments [66].

Here we use the zebrafish somite image reported in [66] as an example to perform somite annotation. We used the iPad prototype system to annotate all of the somites and use the total number of somites during zebrafish embryogenesis as a developmental biomarker. As shown in Figure 17, the zebrafish somites (red curves) can be quite easily annotated via the iPad system and the delineation is pretty effective. Similarly, the iPad-based somite annotation process is

much faster than that using mouse on desktop computer. It saves around 30% time in comparison with that using mouse on desktop computer. In the future, the annotated somites have important potential applications in quantitative phenotyping of zebrafish development and in zebrafish-based screening for drug discovery [67, 68].

Furthermore, we envision that the presented iPad bioimage annotation system could be useful in many other bioimage annotation applications, as the multi-touch interface in iPad provides a natural and friendly approach for human-computer interaction.
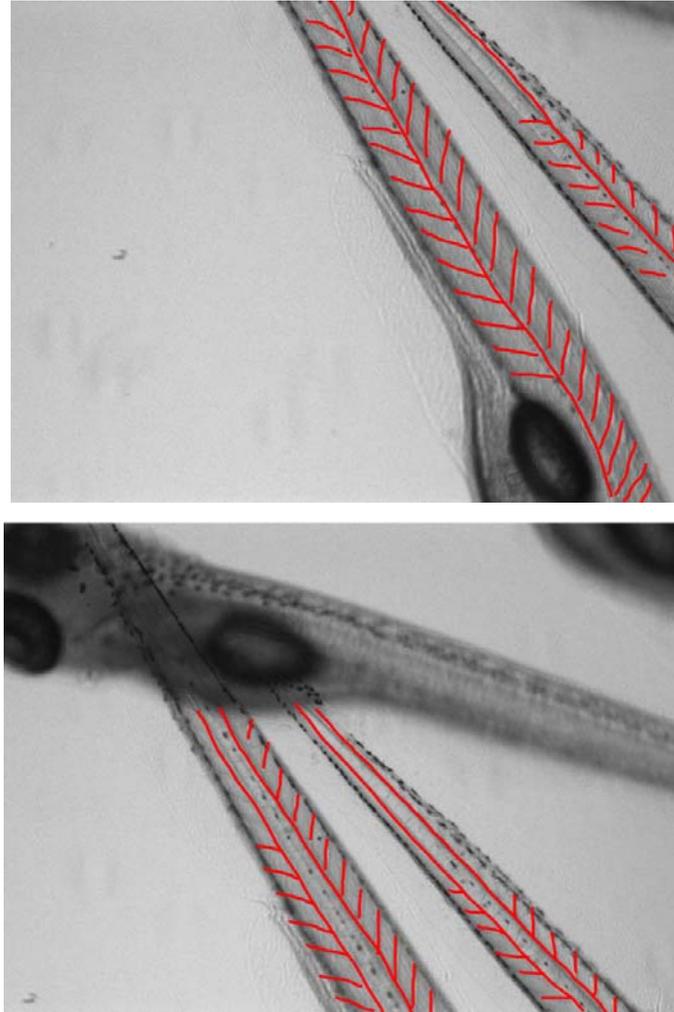

Figure 17 Two examples of zebrafish somite annotation. The somites are denoted by red curves. Figure courtesy: Dr. Weiming Xia

CHAPTER 5

CONCLUSION

iPad is equipped with user-friendly multi-touch screen, large and high resolution display, fast Internet access and powerful processing unit. In this paper, we designed and implemented two prototype systems to demonstrate the feasibility of using iPad for interactive bioimage processing and management. By applying our two prototype systems on a variety of bioimages, for example, neurite images, zebrafish microscope images, cell images, and somite images, we extensively evaluated the prototype systems. The results from our qualitative and quantitative analysis have shown that iPad based segmentation and tracing is much faster, more natural, and more accurate than interactive segmentation based on using mouse on desktop computers. In particular, the more complex bioimage data is when using iPad, the more time the user will save. Based on our preliminary work above, we believe that iPad is a powerful platform for general-purpose bioimage informatics applications.

In comparison to smartphones such as iPhone, iPad has significantly larger touch screen, which is much more convenient for bioimage management and visualization. The characteristics of wide availability, affordable price, user-friendly interface, portability, and ubiquitous connection make iPad a very promising computing and visualization platform for bioimage informatics applications. Notably, the next generation of iPad2 released recently [69] has significantly improved graphics capabilities and dual-core powerful CPU, which will further significantly enhance the feasibility of using iPad2 in bioimage informatics applications such as

3D volumetric bioimage processing, 3D surface rendering and processing, and semantic image annotation.

Finally, iOS products such as iPhone and iPad are already widely welcomed by average biologists in the community (e.g., [27]), and dissemination of iPad-based bioimage informatics tools should be straightforward. To conclude, we envision that iPad will open up numerous applications in bioimaging informatics, such as interactive visual analytics and mobile computing for bioimages, in the near future, and these iPad-based informatics applications will significantly enhance the productivity of biological research and discovery.

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