

Automatic Glass-Slide Capturing System for Cervical Cancer Pre-Screening Program

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Abstract: Clinical glass-slide capturing system is becoming an important part of telemedicine, medical database and diagnostic system that involves of microscope and image acquisition device which is digital camera. One of the areas of interest is slide capturing system for cervical cancer pre-screening program. An automated microscope with FireWire digital camera will be used to acquire the image and store in digital lossless format. The capturing procedure involves of 100X and 400 X magnifications that are necessary for further diagnosis actions. The system that comes with software and hardware parts also provides image retrieval or displaying facilities for ease of user who are pathologist and cytologist to perform the screening program.

Keywords: Glass-slide capturing, cervical cancer screening program

INTRODUCTION

Automatic glass-slide capturing has been used widely in various fields, including medical field. The main objective is to automate the capturing process besides providing image retrieval or displaying facilities. The images that have been acquired are stored inside computer memory so that it can be referred later. In the current study, the automatic glass-slide capturing system is used for cervical cancer pre-screening program, which focus on ThinPrep[®] slide. The system consists of automated microscope, Leica[®] DM LA and FireWire digital camera, PixeLINK[®] PL-A662, that come with Application Programming Interface (API) for software development and interfacing task. The whole system is built on Borland[®] C++ Builder environment. The system is also capable to display the images that have been captured in various ways that will make the system become much easier to use.

Overall Diagram: An overall diagram of automatic glass-slide capturing system is shown in Fig. 1. From

the figure, it can be noted that the system has 3 main components; FireWire digital camera, automated microscope and desktop computer. Both of FireWire digital camera and automated microscope are connected to the desktop computer, while the camera is attached physically through C-mount adapter at the trinocular of the microscope, for image viewing and capturing.

Firewire Digital Camera: The digital FireWire camera, PL-A662 is a colour digital camera from PixeLINK[®]. It uses FireWire (IEEE-1394) digital bus protocol to streamline capturing, digitization and processing of large image streams. It is build from flexible and full-featured CMOS image sensor with resolution up to 1.3 mega pixel array^[1].

From the 1.3 mega pixel array of imaging sensors, user can freely define their own region of interest (ROI), which refers to user-defined rectangle that reflects the image resolution captured from the camera. The imaging sensor array is known as sub-window, with maximum width and height of 1280 and 1024 pixels, respectively.

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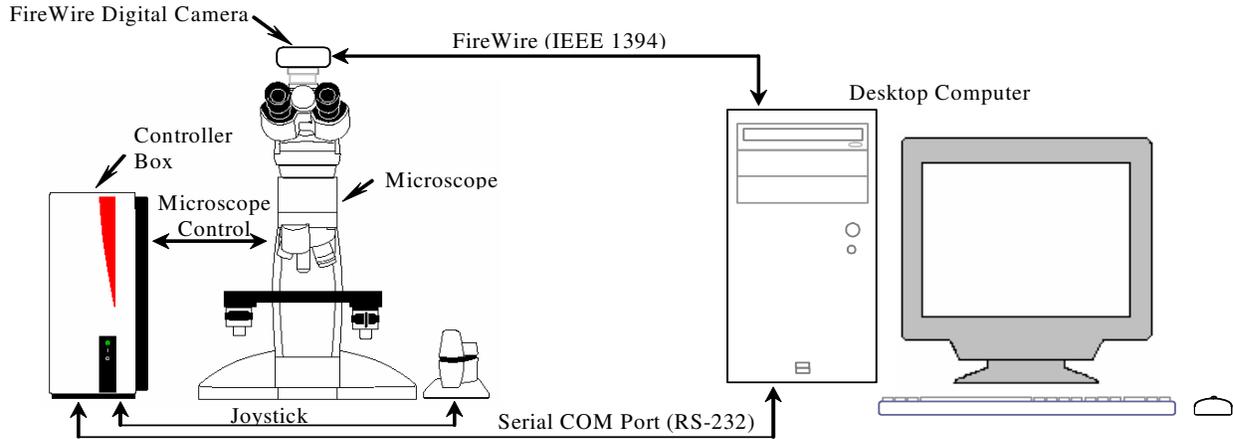


Fig. 1: PC-based Automatic Glass-Slide Capturing System for Cervical Cancer Pre-Screening Program



Fig. 2: PixeLINK® PL-A662 FireWire Digital Camera

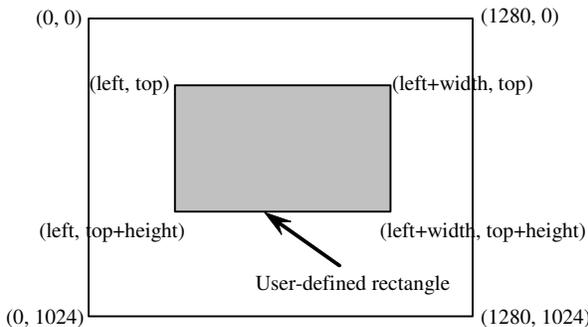


Fig. 3: Sub-window Area and User-Defined Rectangle

Figure 3 shows sub-window area and user-defined rectangle^[2]. In the current study, the user-defined rectangle is selected as 640 x 480 (width x height) for fast processing time.

The PixeLINK® API functions may be called from a C/C++ program or a Visual Basic program^[2]. The API is a Dynamic Link Library (DLL) that provides a set of functions for:

- setting imaging parameters,
- viewing images, and

- capturing frames and storing them as still image or video files.

In the current study, Borland® C++ Builder is used. The DLL provided by PixeLINK is converted into Borland® import library and linked to the program. Header files (PimMegaApiUser.h and PixeLINK.h) are also included in the program. To use the camera, it has to be initialized first before setting up camera parameters such as camera clock frequency and sub-window size^[2]. This is followed by setting up capturing parameters such as gamma correction, saturation and colour gain. The image is ready to be captured after previewing the video in preview window at the program display window. The complete program flow for capturing process of one frame of video stream from the camera is shown in Fig. 4.

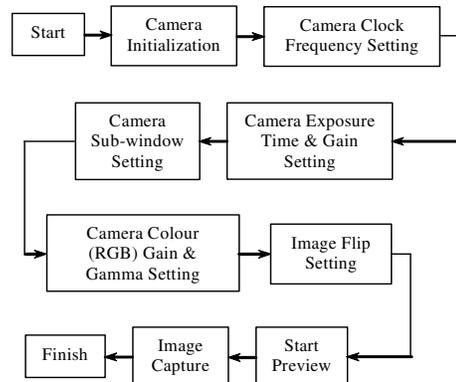


Fig. 4: PL-A662 Program Flow for Single Frame Capture

Automated Microscope: The automated microscope, DM LA from Leica[®] Microsystems, is an upright microscope with fully motorized functions. The microscope is basically similar to a normal microscope except for the automated functions. It has 6 nosepieces for bright field objectives and stage dimension of 234 x 157 mm. with travel range of 76 x 50 mm. The microscope also comes with trinocular that provides attachment with digital camera through C-mount adapter. It uses 100W halogen bulb for illumination and has diaphragm to control the amount of light through the slide of specimen^[3].

The motorized microscope communicates with desktop computer through serial communication port (RS232) for providing full control to the user, automatically. The motorized functions include objective change, stage movements in *x*, *y* and *z* directions and diaphragm aperture. Illumination intensity control is also provided by changing the voltage level for the 100W halogen bulb. All these features can be controlled from joystick or from desktop computer. To drive all the motorized functions at the microscope side, voltage supply for the illumination system, interface for joystick and desktop computer (RS232), controller box is used^[3]. Figure 5 shows the main components for the Leica[®] DM LA automated microscope system.

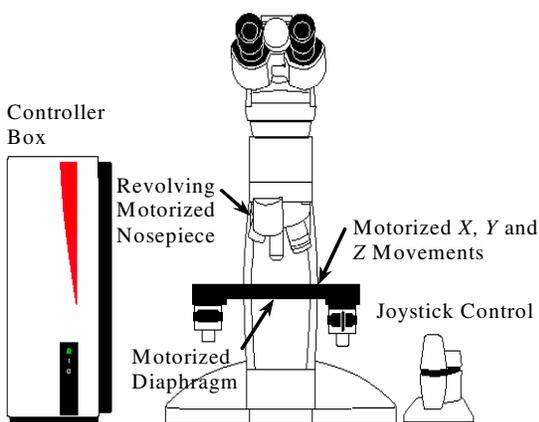


Fig. 5: Leica[®] DM LA Automated Microscope

Basically, to program the microscope to perform certain task is done by sending a predefined command through the serial communication protocol^[4]. Each task is divided into a few groups of components as described in Table 1, as an API for the microscope. The components are the basic entities for the Logical Microscope Mode which represents single microscope functions. For example, to move the stage of the microscope in *X* direction, the component ‘Stg’ should

be used. To achieve that, user has to select a suitable command inside the ‘Stg’ component to move the stage in *X* direction.

Table 1: Logical Microscope Components

| Component | Description |
|-----------|-----------------------------------|
| ‘Len’ | Objectives (lenses) |
| ‘Lmp’ | Lamp |
| ‘Stg’ | Microscope Stage |
| ‘Tur’ | Revolving Nosepiece (turret) |
| ‘Zdr’ | Focus Drive |
| ‘Lgm’ | Includes all the above components |

Leica[®] DM LA API is provided for C/C++ programmer to develop their own software that has the capability to control the microscope. In the current study, Borland[®] C++ Builder is used to take all the benefits inside the API to control the microscope. Leica[®] DM LA microscope will take part in positioning the ThinPrep[®] slide with appropriate focus position by using a suitable lens, while PixeLINK[®] PL-A662 will be used for previewing and capturing the slide, frame by frame, into digital format.

The Automatic Capturing System: In this study, a simple user interface has been developed that consists of single image capturing and automatic slide capturing. The image taken will be stored in a lossless digital format, bitmap (.bmp). The system is capable to displays the images in various ways that make the system become much easier to be used. The objectives of the system are:

1. Providing slide capturing system that can be easily operated by technologist.
2. Imitating the actual pathologist or cytologist screening procedures of the smear slide by providing 10X and 40X magnification capturing.
3. Providing access to the live image of the slide on the screen, different to most of slide scanners in the market.
4. Lessen the time taken to review the slide, especially for tasks that require exploring and/or analyzing complete slides.
5. Providing options to relocate position on the actual slide.

Fig. 6 shows a main user interface for the system. In the following sections, a brief discussion about the system including the image capturing and displaying facilities will be carried out in detail.

Single Image Capturing: Single image capturing process requires the user to position the slide to the specific region of interest on the slide, manually. This tool is necessary when only certain portion of the slide or only a few cells samples need to be analyzed for

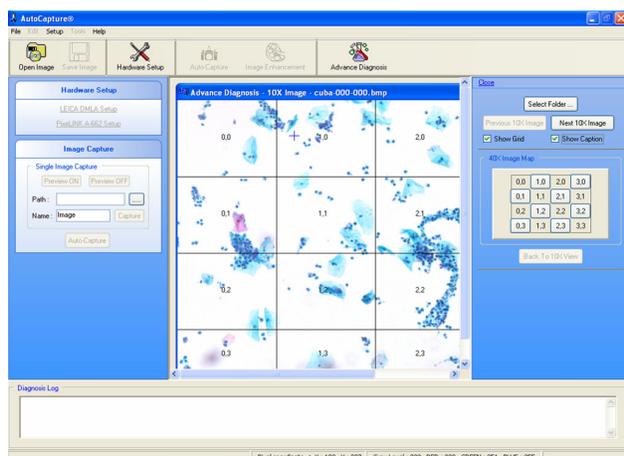


Fig. 6: Automatic Glass-slide Capturing System Main User Interface

further examination. User interface for single image capture is shown in Fig. 7.

User is required to start the previewing process to identify the region of the slide to be captured. The 'live preview' can be monitored in the same display panel of the desktop computer.

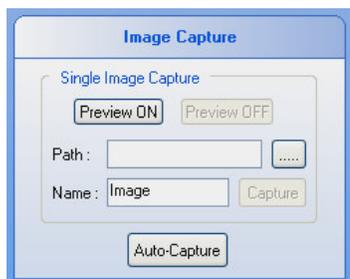


Fig. 7: Single Image Capture User Interface

Automatic Slide Capturing: Automatic slide capture performs capturing for the entire slide of ThinPrep® for the area that contained specimen. The size of the slide is 25.4 x 76.2 mm.^[5] The cells from ThinPrep® are located within circular area of 22 mm. diameter of the slide^[6-7]. Slide label or slide name is located beside the circular area. Figure 8 shows the ThinPrep® slide diagram.

Normal lenses requirement for cervical screening task are high quality lenses with 10X and 40X magnification^[8]. Lens with lower magnification is used to scan and screen for groups of abnormal cells whereas lens with higher magnification is used to see the detail characteristic of the abnormal cells. Usually the process of screening starts with lower magnification and then followed by higher magnification. In the current study,

these two types of lenses are used; both are from Plan Apochromat type. Both of magnifications capturing types are provided; 10X magnification, and followed by 40X magnification. The images will be stored in two folders; one for 10X magnification and the other one for 40X magnification.

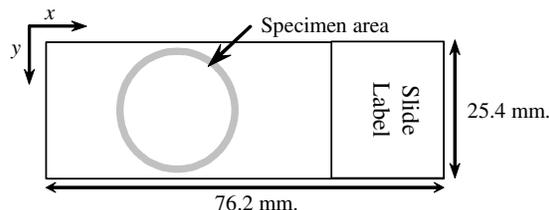


Fig. 8: ThinPrep® Slide Diagram

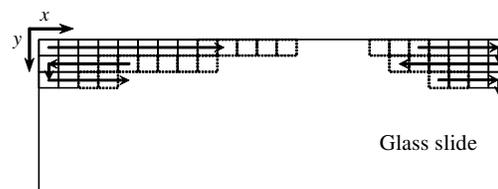


Fig. 9: Direction of Capturing Process on the Glass Slide

The capturing process starts at the top and left most position of the slide. Starting with 10X magnification, the capturing task will continue from left to right (along x axis), frame by frame, until the limit of travel range predefined in Hardware Setup for the automated microscope. The capturing will continue in y direction, followed by capturing from right to left. This process will continue until the entire portion of the wanted area from the slide has been captured, for both of magnifications, 10X and 40X. Figure 9 shows the direction of capturing process on the glass slide.

During capturing process, there are some header files that will be created automatically by the system. The header files will record the total number of images captured, images filename in capture order and its grey level value.

The images' filename that will be saved in the desktop computer memory is consists of slide number or slide name, x and y coordinate. Figure 10 shows the image filename format.

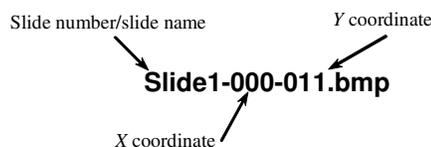


Fig. 10: Image Filename Format for the Automatic Glass-Slide Capturing System

In between of each slide capture, the stage of the microscope will move to load position; a position where user can unload the captured slide and change with a new one. The stage will then automatically move to the start position of the capturing process (top and left most), in focus position. Figure 11 shows the flow in automatic slide capture process.

Image Retrieval/Displaying: Image retrieval or displaying is a function in the automatic slide capturing system that provides various ways of displaying the image captured. This function allow multiple image displaying through thumbnail preview and also in some other ways to make the system become much easier to use for further diagnostic purpose.

In the following sub-section, 4 methods of image displaying inside the system will be presented. The methods are; thumbnail based on capture order, thumbnail based on most suspected area, 10X – 40X map and also inter-image cells.

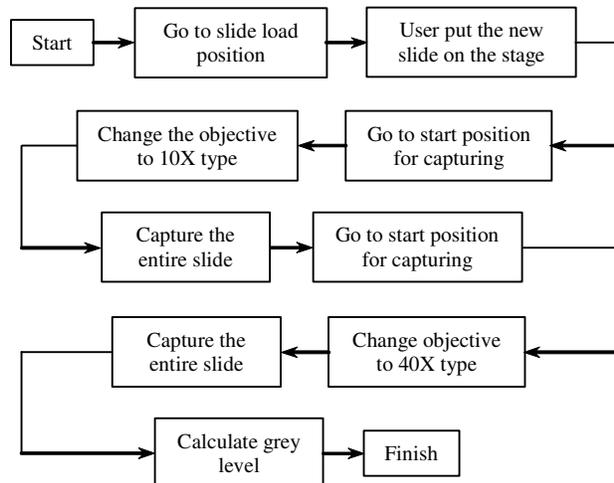


Fig. 11: Flow Chart of an Automatic Slide Capturing System

Thumbnail Based On Capture Order: As discussed in the previous section, slide capturing start at top and left most of the ThinPrep® slide, and move along the x axis to the right. Then, on the new row (an increment in y axis), the process will be repeated from right to left. This process will continue until the entire portion of the wanted area from the slide has been captured.

The 40X magnification images can be displayed inside the thumbnail panel on the system. The system will sort the images as sorted inside the header file,

which are in capture order. The thumbnail with images sorted in capture order is shown in Fig. 12.

THUMBNAIL BASED ON MOST SUSPECTED AREA

Cancerous cell in cervix will have an increase in nucleus-cytoplasm ratio as a result from mitosis process that occurs only in nucleus but not in cytoplasm [9][10]. In images that contained a group of cervical cells, this fact can be used to predict an occurrence of cancerous cells among the images captured. The image that has high nucleus-cytoplasm ratio will be placed on top position of the thumbnail panel to be screened. This will lessen the time taken looking for abnormal cells since the occurrence of the cancerous cells is quite rare in most cases. This facility is provided in the system as shown in Fig. 13.

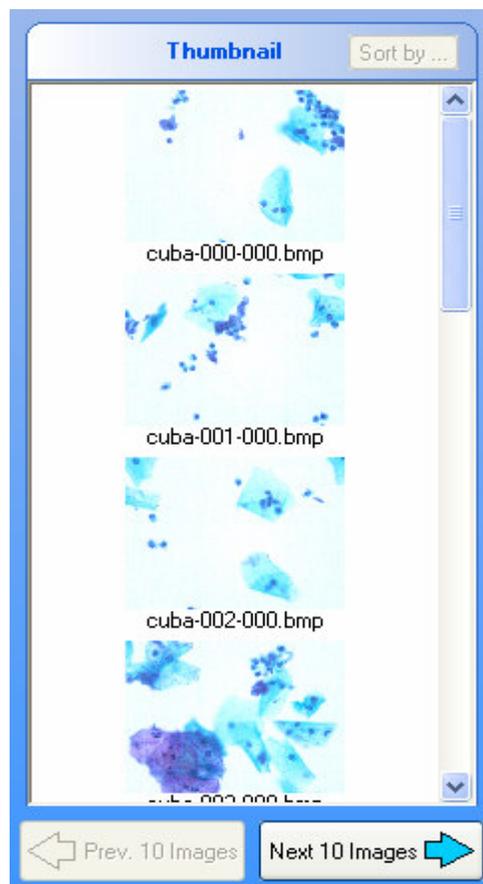


Fig. 12: 40X Images Sorted in Capture Order



Fig. 13: 40X Images Sorted in Ratio of Nucleus-Cytoplasm Areas

10X-40X Magnification Map: Normal lens requirements for cervical screening program are 10X and 40X magnifications [8]. The 10X magnification lens is used to scan and screen for groups of abnormal cells whereas the 40X magnification lens is used to see the characteristic of the abnormal cell. Usually, the process of screening starts with the lower magnification lens and then followed by the higher magnification lens. In the current study, the actual pathologist or cytologist approach to screen the ThinPrep® slide is adopted. Pathologist or cytologist usually examines the slide under 10X magnification, looking for suspected area on the slide. Upon finding the area, they may want to see the slide under 40X magnification for examining the cells' nucleus and cytoplasm.

In the system, the captured 40X and 10X magnification images are linked together through mapping technique. The technique enable user to switch from 10X magnification image to the 40X

magnification images, and vice-versa. The 10X magnification image is divided into 16 equivalent 40X magnification image. Figure 14 shows 10X-40X magnification map for the system. At the right panel, a group of buttons for 40X magnification images browsing are provided along with 10X magnification image browsing buttons.

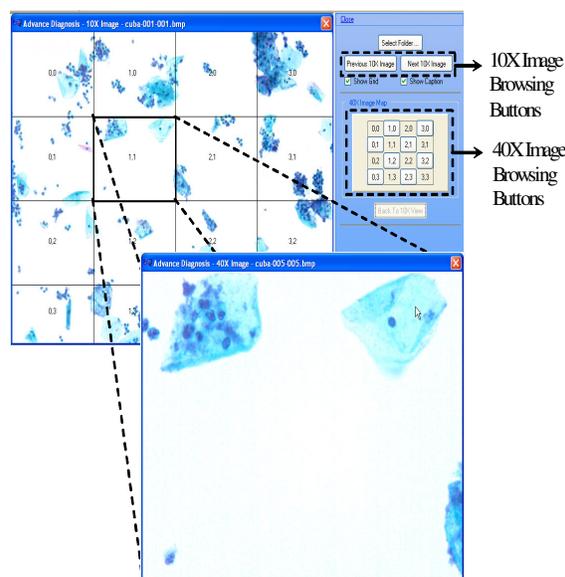


Fig. 14: 10X-40X Magnification Map

During screening process in the system, user may start diagnosis by browsing through the 10X magnification images, looking for suspected areas for abnormal cells. The user may then uses the 40X image browsing buttons to view the 40X magnification image of certain portion of the 10X magnification image. Here, the role of microscope is replaced with software system that capable to switch between these two types of magnification without having to put the slide on the microscope stage.

INTER-IMAGE CELL DISPLAY

In an image from 40X magnification type, the occurrence of partial displayed cell is always happen. In such case, to fully view the cell, the actual slide is needed. User needs to find the cell manually, capture and stored in computer memory. In this system, the cell can be fully displayed in the same window, without any other capturing process. The feature is called inter-image cell display. Inside, the neighbour images are cached and can be displayed next to the original image.

The inter-image cell display is highly depends on the accuracy of the automation part of the automated

microscope. It also relies on the filename system of the 40X magnification images captured. Once used, the system cached the original image along with its eight neighbour images to be displayed according to what user needs. Figure 15 shows the user interface for inter-image cells display.

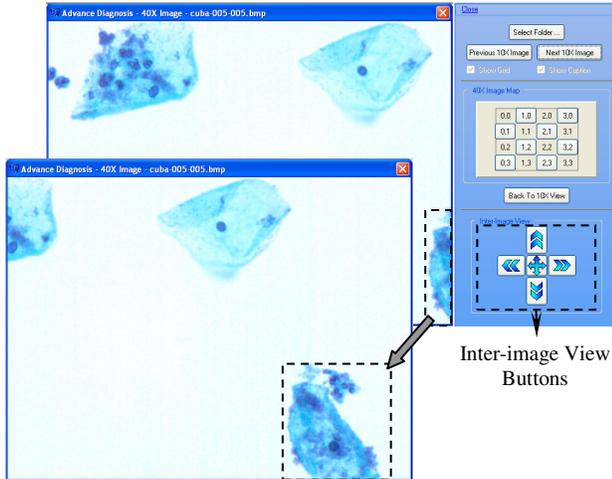


Fig. 15: Inter-image Cell Display

CONCLUSION

In this article, the automatic glass-slide capturing system is proposed and discussed. The system employs the digital FireWire digital camera, PL-A662 and the automated microscope, Leica DM LA as the hardware parts to automate the capturing system. The overall system is built on Borland C++ Builder environment. The hardware parts communicate with the desktop computer through Application Programming Interface (API), which enables direct control from Borland C++ Builder. The system also includes image retrieval or displaying facilities for ease of user who are pathologist and cytologist to perform the screening program.

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