Development of Confocal Imaging System for Wafer Inspection

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개발 웨이퍼 검사위한 Confocal 이미징 시스템의 개발

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Abstract

Confocal Imaging System is an essential machine for a wide range of inspection wafer. For concurrent and fast acquiring the image data of four channels, the new image acquisition system used the protocol of camera-link standard with the full mode of configuration in interconnection with a frame grabber integrated in a computer , which is popularly used for many cameras, so the programming environment of image processing is optional such as Visual C++, Matlab. In addition, many conventional methods were coordinately used for contribution to build the high quality of images for precise processing analog signals of PhotoMutiplier Tubes(PMTs), accurate control of scanning device, sensitivity of PMTs, and laser source. The prototype of new image acquisition system, could meet the goal of development, it is used in LSCM for high content screening to investigation the processes of elements of living specimens at the same time by simultaneous grab image data on 4 PMTs channels.

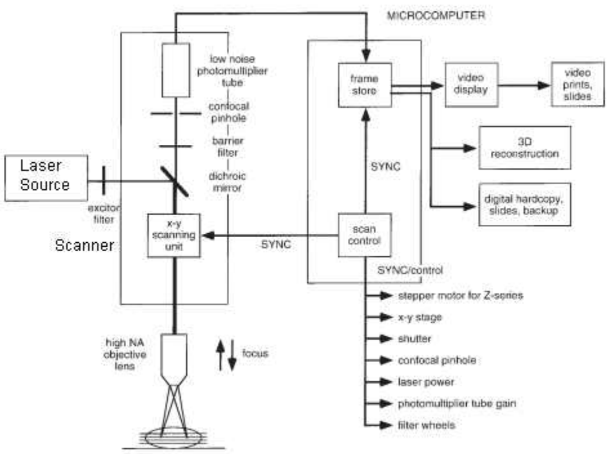
1. Introduction

The CIS(Confocal Imaging System) is widely used in many applications such as Semi-Conductor inspection and Biological field. Through the use of multiply-labeled, different probes can simultaneously identify several specimens. The basic concept of a confocal microscopy was invented by Marvin Minsky, who has built a working microscope in 1955 with the goal of imaging neural networks in unstained preparation of living brain, and all of the modern confocal imaging systems employ the principle of confocal imaging that he patented in 1957 . In Minsky's original confocal microscope the beam was stationary and the specimen itself was moved on a vibrating stage. However, movement of specimen can cause of distortion and wobble of imaging, and is also impossible to perform various manipulations such as microinjection of fluorescently labeled probes. Continuing this area in company with advances of electronics and optics, the components of confocal microscope were developed such as a scanning unit (galvanometer (Y-axis) and resonator (X-axis)), powerful lasers, fiber optics, detectors (PMT) and image acquisition system, computer processing speed and so on. The Image Acquisition system functions formation of confocal images by driving a scanning device (a vertical scanning galvanometer and a horizontal fast scanning resonator), by adjustment the gains of PMTs, by processing the synchronous signals, which frame an image of the specimen, on the output of scanning device, by digitally processing the analog signal that is directly proportional to brightness of incident light striking on PMT (in Image Acquisition Section in Fig.1). The quality of an image of CIS is affected by four principal factors: spatial resolution, resolution of light intensity, signal-to-noise ratio, and temporal resolution [5]. As a result, the image acquisition system of CIS has to work in combination condition for having optimal factors, so it is had better particularly designed just for a CIS.

To develop CIS, we have proposed the method to design the high speed image acquiring system for increase the rate of images per second by simultaneous imaging of four fluorescent color channels (Blue, Green, Red, and Yellow) with high resolution of image data up to 16 bits, and automatic accurate control its peripherals, which contribute to form images of a specimen, such as a scanning device, PMT, and laser source by program on a computer for having the image speed is 30x4 frames per second.

2. Materials and Methods

2.1 The overall confocal microscope



[Fig.1] The block diagram of LSCM

The CIS consists of Microscope Section and Image Acquisition Section. A typical Microscope Section contains laser excitation sources, fluorescence filter sets and dichromatic mirror, which is an accuracy color filter used to selectively pass light of a small range of colors while reflecting other colors, a scanning device (which includes galvanometer scanner and resonance scanner), variable pinhole apertures for generating the confocal image, Acousto-Optic Tunable Filters (AOTF) for simultaneously modulating the intensity and wave length of multiple laser lines from one or more sources. The emission filters for selecting the emission wave length of the light emitted from the specimen and to remove traces of excitation light, and detectors (PMTs) tuned for different fluorescent wavelengths.

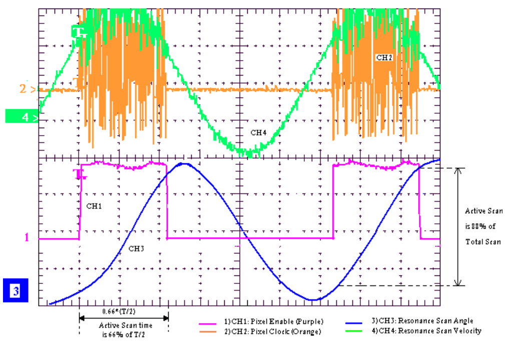
The scanning device with the vertically scanning galvanometer (Y axis) and horizontally fast scanning resonator (X axis), is responsible for rasterizing the excitation scans, as well as collecting the photon signals from the specimen that are required to form and to assemble the final image. The image can be zoomed with no loss of resolution simply by decreasing the region of the specimen that is scanned by the galvanometer mirror and resonator mirror.

2.2 The image acquisition system

The image acquisition section takes an important part for making a good image of the specimen. It has two main block functions: the one is an image acquisition block, which frames an image on a computer by processing the synchronous signals made by scanning system, for making a shape of the image (for example: the image size 512x512 means that there are 512 pixels in a line and 512 lines in a frame), and by constant dyeing a color, which has an intensity directly proportioned to striking light on PMT, on each pixel of the image frame. There are five PMTs (PMT1, PMT2, PMT3, and PMT4) in the system. Each PMT is the most sensitive with the small range of wavelength of color light such as Red, Blue, Green, Yellow, Violet,... and is a single channel to form an image of the specimen, so the specimen would be observed either in four separate images with correlative colors or in an image of mixing four PMTs channels. The other is Control Unit, which makes analog and digital signals to precisely control scanning device, PMTs and other peripherals.

2.3 Method to develop the Image Acquisition System Processing the synchronous output signals of the scanning device

A scanning device usually consists of a resonance scanner, which horizontally fast scans forming 512 pixels a line, and galvanometer scanner, which vertically slowly scans forming 512 lines a frame. The resonance active scan is 88% of total scanning angle (a linear area) correlatively with 33% of a scanning period (Fig.2a). Otherwise, light beams would be in the out-of-focus area (eliminated). Consequently, a pixel enable pulse (CH1 on Fig.2) is made by a velocity signal (CH4 on Fig.2a), which is differential of an angle signal (CH3 on Fig.2), as mean the same period and its duty as the active scan time. Pixel clock pulses are gated by a pixel enable pulse. These pixel clock pulses (CH2 on Fig.2) configure the pixels per line on the image of the specimen, for instance, there are 512 pixels clock pulses gated by pixel enable pulse, so there would be 512 pixels per line on the image frame (Fig.3B).



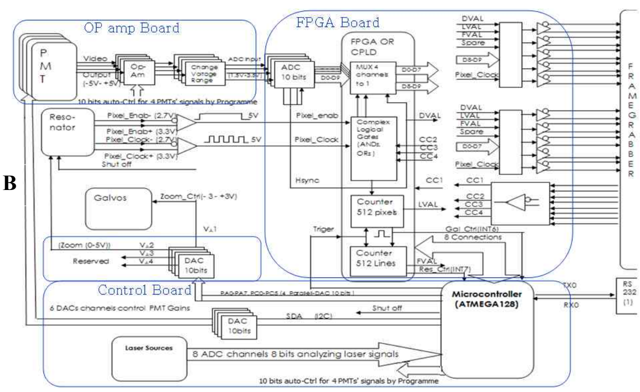
[Fig.2] The interrelationship between a scanning angle signal, a velocity signal, Pixel Clock Pulse and Pixel Enable pulse in the resonance scanner.

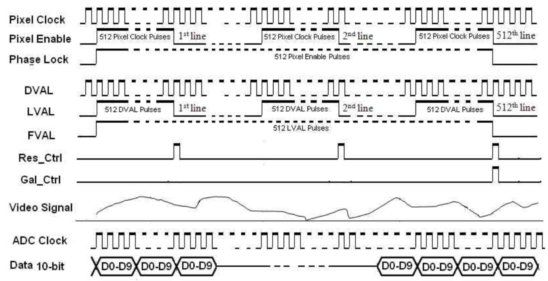
To process the synchronous signals from scanning device, The FPGA chips of Altera Corporation in company with the programming environment Quatus II were selected. All triggered events of Pixel Clock, Pixel Enable, Phase Lock pulses such as falling edges and rising edges were captured based on Pixel Clock, which is the global clock (the FPGA block diagram on Fig.2). By using the counters and logical complex block, The Pixel Clock pulses are gated by Pixel Enable pulses with the constant number of pixel a line, the Pixel Enable pulses are gated by Phase Lock Pulse to form the number of lines per an image frame (the timing chart Fig.2). The function blocks like counters and logical complex block of FPGA chip can easily be programmed by using programming languages such as text (VHDL) or graphic or timing chart, so the firmware inside FPGA chip can compensate few deficient pulses, in the case that scanning device works incorrectly, to fix enough the number of pixel per line and the number of lines per image frame. The corrected synchronous signals on outputs of FPGA were changed to enable signals of standard camera-link such as: DVAL, LVAL, and FVAL. The Pixel Clock is frequently multiplied by 7 times, and then it samples the DVAL, LVAL, FVAL and data of a pixel (up to 12 bits) to packing standardly and send to frame grabber, which is being used standard camera-link protocol. These functions could easily be implemented by the single channel Link chip family such as DS90CR287 converting to the LVDS signals.

Making the analogue controlling signals for scanning device, and PMT and other peripherals

As the above analysis of the signals of the scanning device, the synchronous signals are very important to frame an image on a computer.

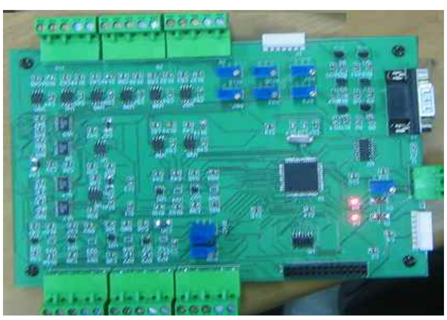
B



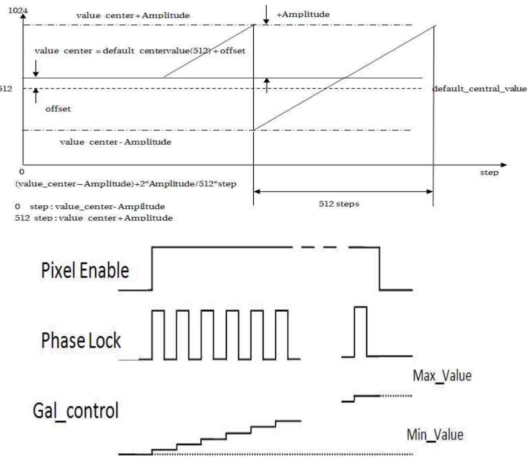


[Fig.3] (A) The block diagram timing chart of signals in the image acquisition system prototype. (B) The timing chart of signals in the image acquisition system prototype.

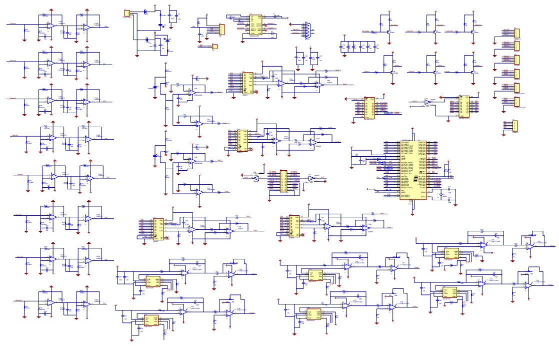
However, these signals are closely relative to the controlling signals on input of scanning device such as the galvanometer control signal (Gal_control), the resonance control signal (Res_control), and the enable signal announced in a datasheet of the scanning device (Fig.4 a). Especially, the value of the amplitude of Res_control directly affects to Pixel Enable, Phase Lock, and frequency of Pixel Clock, and the Gal_control controls the reflecting region of light beam focused in Pinhole and PMT. Therefore, the requirement of these signals is accurate. The microcontroller and parallel high-speed DAC were proposed for making controlling signals for the scanning device. In the figure 4d the method to make a flexible Gal_control was proposed, then this signal is increased (decreased) a voltage step of 2A/512 (V) at each falling edge of LVAL signal during the FVAL pulse from -A+a to A+a (A+a to -A+a) (A is its amplitude, and a is the central value of Gal_control signal with ranges 0<A<3 and -A/2<a<A/2). A can be changed from microcontroller by using another DAC, whose analog output signals supplies to reference voltage of the DAC making Gal_control



signal, a is changed by firmware in a microcontroller. So that, its amplitude (A) and central voltage (a) could be changed in the voltage range (0-3V) by commands from a computer using the protocol of UART (universal asynchronous receiver/transmitter) communication in the prototype.



[Fig.4] (A) The method to make Gal_control signal. (B) The form of the required Gal_control signal.



[Fig.4] The schematic circuits of controller board and the PCB board.

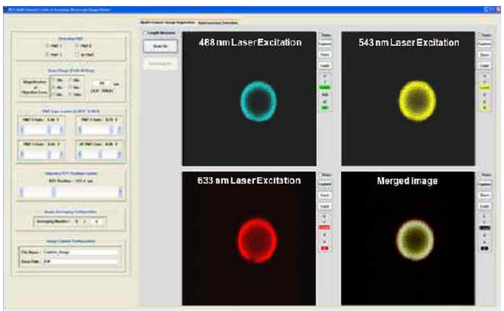
Countermeasure of noise and disturbance signals in the image acquisition system

In fact, the image acquisition system is very complicated because of mixing analog and digital parts. Thus, there are many noise sources such as: a power source, a scanning device, noise of digital parts to analog parts, to influence on image acquisition system and also the quality of image. However, all signals (Controlling signals for scanning device, gain control signal of PMT, and video signals) require to be extremely accurately processed, for instance, commonly the noise level of Gal_control, Res_control, and controlling signals of PMT gain is less than 10 mV, and video signal is less than 5 mV.

Correspondingly, the method of reduction noise was used, integrating some techniques such as isolation of analog and digital parts by using the inductors, ferrite beads, and capacitors, and using high order low-pass or band-pass filter, which has the bandwidth fitting with the frequency spectrum of the signals.

3. The Results

The acquired images could meet the goal of high peed acquiring system for high content screening. The photon signals of four PMTs channels were parallely processed to store in the memory of a computer supporting image data for discovery the cellular properties in HCS system. By using the specimen on well-plate with four laser excitation sources, the images on four screens were displayed .



[Fig. 5] *The image of the focal check (invitrogen)*

5. Discussion

Actually, the resonant scanner is only active in one-direction, the supplied device driver do not support for bi-diectional scanning. Therefore to increase the rate of frames, the backward direction of resonant should be considered.

6. References

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