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Scanning and Image Reconstruction Techniques in Confocal Laser Scanning Microscopy

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1. Introduction

Taking advantage of the laser scanning methodology, confocal microscopy has been widely applied in cutting-edge biological research for its three-dimensional, high-resolution imaging capability (Pawley 1995). The principles of confocal were put forward by Marvin Minsky in 1955 (Minsky 1988), yet the experimental demonstration was not accomplished until 20 years later by Cremer brother (Cremer, Cremer et al. 1978), and Brakenhoff et al. (Brakenhoff, Blom et al. 1979). In confocal microscopy, one or more focal spots are scanned relative to the specimen, to extract the signal from the focal spots. The 3-D image can then be reconstructed digitally. Contrary to mechanically scanning the specimen, which is relatively slow as the specimen has to be moved, the laser provides fast scanning and imaging speed for confocal microscopy and therefore is routinely used in commercial state-of-the-art confocal systems. Several laser scanning mechanisms have been employed in confocal microscopy:

1. single spot laser scan with galvanometers;
2. single spot laser scan for real-time imaging and endoscopy;
3. line scan confocal with a line detector or a 2-D detector;
4. multiple spot scanning with 2-D detectors.

The single spot galvanometric scan is the most straightforward laser scanning mechanism, which provides an imaging speed of ~1 frame(s) per second (fps) for a typical 512 x 512 image, and is widely used in many confocal laser scanning systems. However, the relatively slow scanning speed of the galvanometer commonly used in a confocal laser scanning microscopy can dramatically limit the system performance in scanning speed and image quality if the data collection is simply synchronized to the galvanometric scanning. In Section 2 we discuss the command and data processing techniques for galvanometric scanning, for example, pixel delay and interlace line switching can be applied on bi-directional scans to cancel aliasing; pixel binning and/or image average can be used to improve the signal-to-noise level; Acquire-On-Fly scanning can be applied when the signal command is a limiting factor for the imaging speed.

Due to the limitations placed on maximum galvanometer scanning speed, real-time confocal microscopy relies on the fast scanning mechanism on its fast axis, such as a resonant scanner or rotational polygon mirror. This is discussed in Section 3. The resonant scanner runs at a fixed frequency with variable amplitude, and scans a temporal sinusoidal pattern.

Therefore, the pixel reconstruction has to be processed to correct the sinusoidal scan waveform and the bi-directional scan. Contrary to the resonant scan, a rotational polygon mirror provides a uni-directional, constant velocity scan, although the scanning angle is fixed the number of sides on the polygon. Fast scanning can be found in several versions of novel commercial confocal laser scanning microscopes. To miniaturize the size of the conventional scanning head, MEMS-based scanning mirrors are employed as well as vibrating optical fibers for fast endoscopic scanning.

In Section 4 we describe line scan confocal microscopy, in which a cylindrical lens is used to focus the beam to a line on the specimen. The line is scanned with a galvanometer to reach video-rate imaging. Signals from the specimen can be detected by a line detector, or be re-scanned and viewed by naked eye or a 2-D detector. In such a system, the signal collected from the line detector has to be synchronized with the scan to form a confocal image.

Multiple spot scanning is an alternative scan geometry that employs multiple focal spots to scan across the specimen simultaneously. As discussed in Section 5, spinning disk scanning is the most widely used multiple spot scan mechanism. To cover the specimen, a spiral geometry is used in the spinning disk to arrange the microlens array, which form focal spots, and the pinholes. Unlike other confocal laser scanning modalities, the 2-D CCD detector system can be used directly in multiple spot spinning disk scanning.

2. Galvanometric single spot laser scanning

The principle of confocal microscopy is illustrated in Fig. 1. In traditional wide-field imaging microscopy, the sample is illuminated uniformly and a 2-D detector is employed, such as a film, a CCD or CMOS sensor, or the retina of the eye. This imaging mode lets us see clearly when the target falls within the depth of field, while the targets out of the depth of field are blurred but still contribute intensity to the detector (see Fig. 1a). As a result, we cannot get 3-D information from this configuration. In contrast to wide-field imaging, confocal microscopy is able to achieve this from point detection. From Fig. 1b we can see that only

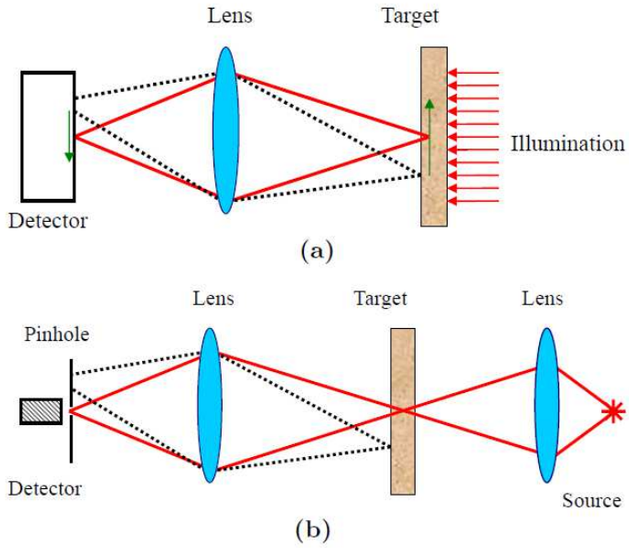


Fig. 1. Different types of microscopy: (a) wide-field microscopy; (b) confocal microscopy.(Xi, Rajwa et al. 2007)

one point is illuminated and the signal from this point is exclusively collected. Thus, through the relative movement of the detecting point and the target, 3-D information can be collected. The axial scan can be achieved by moving the objective or sample, while the lateral scan can be done by either the movement of the sample (stage scan), or the deflection of the angle of the laser incidence (laser scan). In this chapter, we will focus only on the lateral scan with laser scanning mechanisms.

Historically, a mirror galvanometer is used as mechanical meter that senses electric current and moves the direction of the mirror. The mirror reflects a beam of light, which projects onto a meter, and acts as a pointer to read out the electric current. In CLSM, the galvanometer is used in reverse, that is, to deflect the light to the specific angle driven by a known current. Galvanometric scanning is the most widely used scanning mechanism, and takes advantage of its accurate scanning across the sample. The most straightforward way to control the galvanometer is that a position (X,Y) is sent to the galvanometer, usually in a raster mode, and the image intensity data is read from the detector point by point.

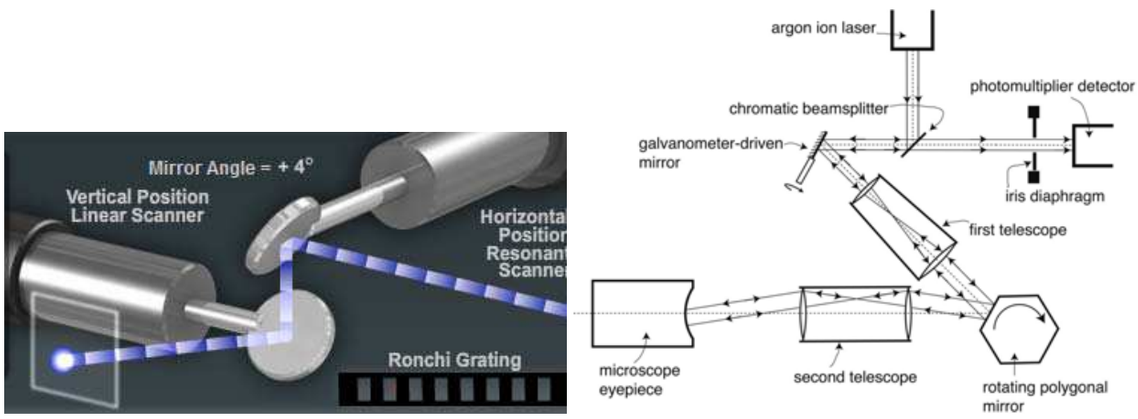


Fig. 2. Geometry of the scaning mirror configuration: Left: perpendicular galvanometers placed close to each other (http://www.microscopyu.com/tutorials/flash/resonantscanning/confocalresonantscanning/index.html); Right: telescope conjugated scanners (Amos and White 2003) Image courtesy of Elsevier.

Fig. 3 schematically illustrates the configuration of a CLSM. A scanning lens is employed to form a telescope; placing the scanning galvanometer at the entrance pupil will efficiently guide the light through the exit pupil of the telescope, where the objective is located. The scanning raster is generated at the back focal plane of the objective-tube lens. The scattered or fluorescent light signal from the objective goes back to the galvanometer and gets descanned, then is detected by a point detector, such as a photo-multiplier tube (PMT).

To realize a XY plane frame scan, the spot is generally scanned in a raster mode similar to that of an analoge television. There are two methods to deliver the light: (1) using two opposing mirrors rotating in perpendicular directions, one for a fast (X) line scan and the other for a slow (Y) frame scan. The two scanning mirrors are located close to each other, and a telescope is used to deliver the scanned beam to the back aperture of the objective. The conjugating plane of the objective aperture is usually chosen to lie in the middle of the scan axes. Because the beam is scanning across the objective, the beam size should be larger than the aperture of the objective so as to always completely overlfill it during scanning. An alternative scheme (2) is to use a 1:1 telescope between the two scanning mirrors located at

the entrance and exit pupil of the telescope, and then use another telescope to deliver the beam to the back aperture of the objective. In this scheme, the light can always pass the back aperture during scanning, therefore excessive beam expansion is not needed.
In the raster scan method, two scanning modes are generally employed: (1) a bi-directional scan; and (2) a unidirectional scan. Each scanning mechanism has its own pros and cons, as described in the following sections (Chen, Feng et al. 2010).

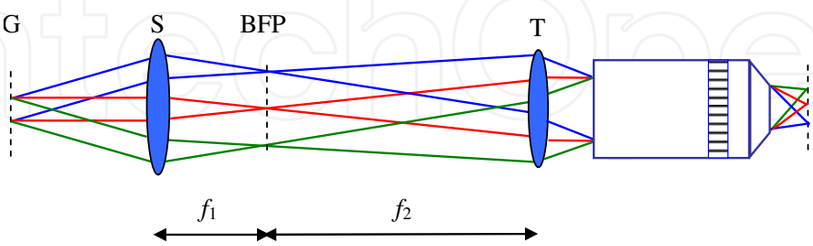


Fig. 3. The configuration of a confocal laser scanning microscopy system. G: Galvanometer; S: scan lens; BFP: back focal plane; T: tube lens; f_1: focal length of S; f_2: focal length of T.

2.1 Bi-directional scanning

In bi-directional scanning the galvanometer scans forward and then backwards at the same speed, and data is collected from both scan directions. The scan control scheme is shown in Fig. 4.

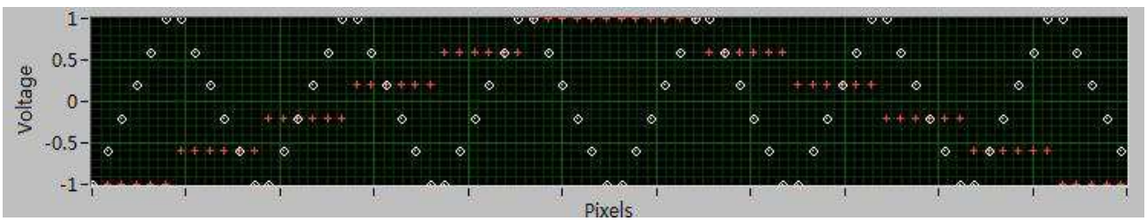


Fig. 4. Bi-direction scanning scheme. White (o): voltage applied onto X axis (fast) versus time (pixel sequence); red (+): voltage applied onto Y axis (slow) versus time (pixel sequence). The data collection of each pixel is synchronized with the scan control (Chen, Feng et al. 2010).

Unfortunately, one of the problems we found when bi-directionally scanning was that the speeds of the forward and backward scans are not simple symmetric functions but are attenuated by the acceleration of the galvanometric control due to the fast scan acceleration. This makes a line shift distortion apparent in the image. To correct the line shift, the pixels in the even (back) line of the forward scan are switched with the odd (forward) scan line of the back scan, so that in each obtained image all the scan lines have the same scan direction (see Fig. 5). It should be noted that the temporal sequence for the image lines is re-arranged, so this is not suitable for tracking particle movement.
A mouse kidney sample was imaged with two-photon fluorescence excitation to demonstrate the effect of line switching (the experimental setup is described in (Xi, Andegeko et al. 2008; Xi, Andegeko et al. 2009). It can be seen that when line switching is applied, the structures labelled with lectin wheat germ agglutin and the filamentous actin in the mouse kidney glumeruli are seen clearly (Fig. 5d); whereas, in the non-corrected image,

these structures are blurred (Fig. 5c). Naturally, the position of the blur is the same for all the three dyes, because the scanning direction is the same when collecting the images for the three channels. An alternative way to cancel the blur is to shrink the image to 25% of its total size; by doing so all the odd (or even) lines are removed; however the pixel number is dramatically decreased, and this can affect either the resolution (if the initial image contains proper sampling) or FOV (if the initial image is taken with twice excessive sampling and then shrinked to half). Note, that for uni-directional scanning (Fig. 9a) the line shift is not presented, simply because the scan directions are constant for all the lines on the image.

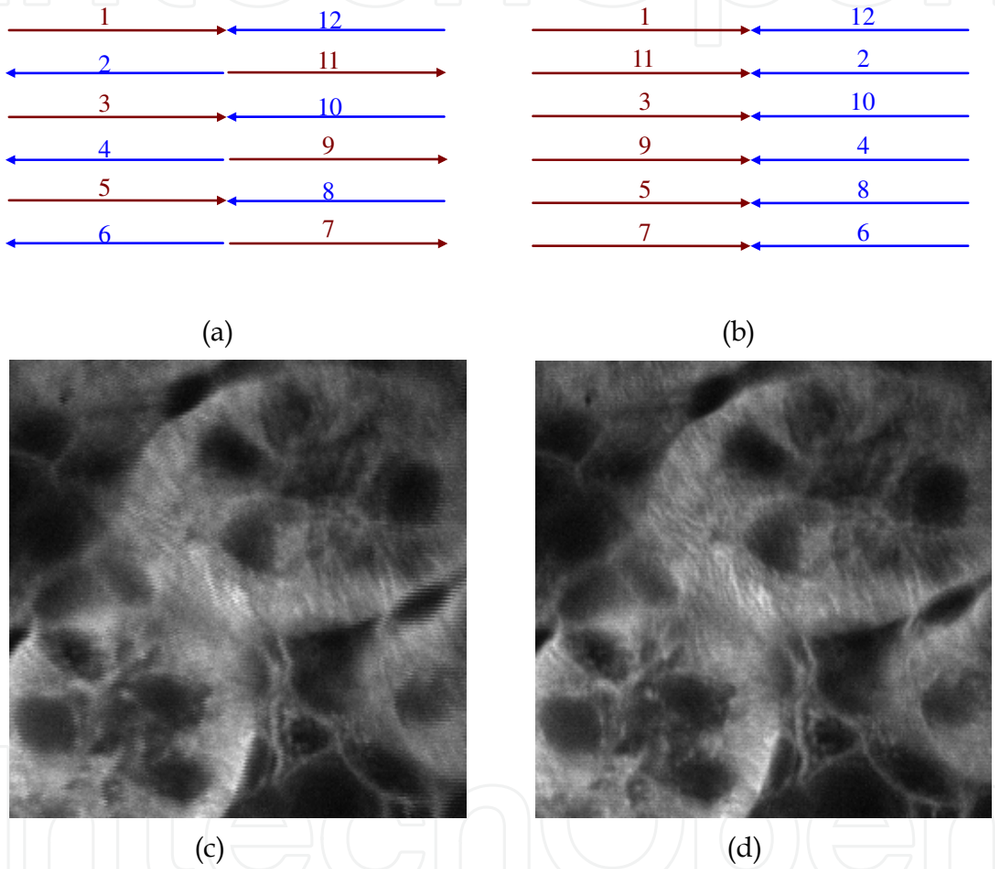


Fig. 5. Bi-directional scanning scheme: (a) before and (b) after line switching; the resulting images of a mouse kidney are shown in (c) before and (d) after line switching (Chen, Feng et al. 2010).

2.2 Uni-directional scan

One of the problems encountred in bi-directional scans is the fact that the torque for the galvanometer is generally not the same between backward and forward scans, which results in image aliasing. Moreover, the lack of constant scan speed results in a slight variation at the edge of the image, where the acceleration of the galvanometer reaches a maximum. As a result, the bi-direction scan yields two images that describe the structure of the biological specimen, but they are slightly different, which makes image averaging inapplicable. In uni-

directional scanning, the backward scan is omitted, therefore, the scan direction on the reconstructed image is always the same (see Fig. 6). The proportion of each full-frame scanning interval that is utilized to actually scan the specimen is referred to as the duty cycle of the system. Due to limitations set by the speed of the galvanometer, the duty cycle of a confocal system is usually 66% at a high frame rate, or 1/2 of the time spent on the forward imaging scan. Because uni-directional scanning can minimize speed differences during forward and backward galvanometer scanning, it is commonly applied in many commercial confocal microscopes, in which a fast backward scan is employed, often followed by a shutter to avoid excessive photobleaching of the sample.

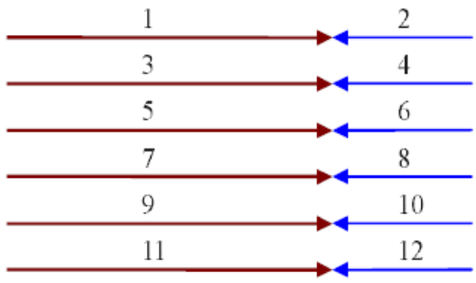


Fig. 6. Uni-directional scan: the brown lines indicate the forward imaging scan, and the blue lines indicate the backward scan, which is not used in imaging (Chen, Feng et al. 2010).

2.3 Pixel delay

Because of the response time of the galvanometer, the commands of the user cannot immediately generate galvanometer movement. Instead, the galvanometer has a fixed response delay if the data acquisition is at the same time as the scan command, and if not properly addressed, leads to a delay in acquiring data and a distortion of the image during image reconstruction. There exists several versions of ImageJ plug-ins, for example ``Correct X Shift'' by Krempp (http://rsbweb.nih.gov/ij/plugins/correct-shift.htm}) that shifts the X distortion by pixels on the odd lines so that the image width gets smaller when shifted, and ``X Shifter'' by Xi (http://rsbweb.nih.gov/ij/plugins/x-shifter.html) that shifts the pixels to the following line so there is a two pixel difference between the neighboring line on each pixel shift. These non-real time image processing tools can only be used to process the collected image, however, real time processing is highly important enabling the confocal user to visualize the structure of the sample properly.

The scheme of shifting pixels is illustrated in Fig. 7. In our program, the image data was collected at the same time as the scan command. To compensate for the response delay, a number of pixels at the front of the data collection were rolled back to the end of the data, resulting in pixel delay of the image (Fig. 8). This corresponds to the delay in data acquisition of the DAQ board and achieves the synchronization of the galvanometer scan and data acquisition. The number of shifted pixels is dependent on the galvanometer hardware. In our system, a response delay of 326.8 μs is applied, which corresponds to 21 pixels for a 256 x 256 image acquisition at 1 fps. It should be noted that due to the pixel shift, several of the last pixels do not contain the real information in the first image. However, as most biological information is located in the central part of the image, this does not create any noticeable effect in the image.

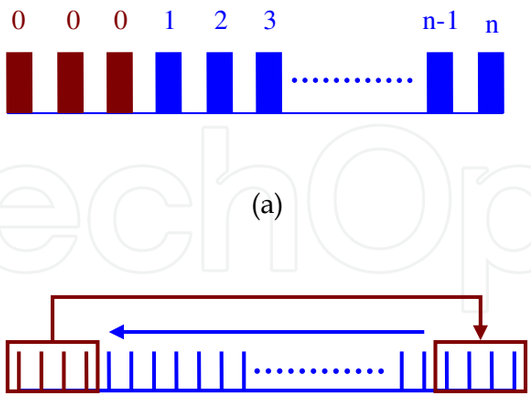


Fig. 7. Response delay of the galvanometer (a) and pixel shift correction method (b). The blue pixels contain imaging data from the sample, while the brown pixels indicate the data collection before the galvanometer responds and thus contain no information (Chen, Feng et al. 2010).

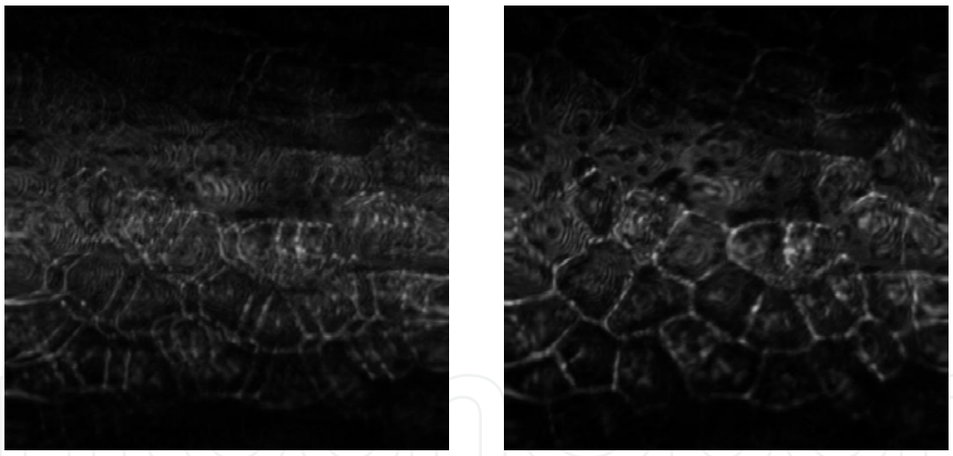


Fig. 8. Effect of pixel delay: before pixel delay correction (a) and after correction (b). Image size: 150 μm (Chen, Feng et al. 2010).

2.4 Data binning

As the data acquisition speed can be much higher than the scan command, multiple data acquisition can be performed with data binning to optimize the SNR. When the image is reconstructed, the software uses a simple arithmetic average to bin the data and applies the average value as the intensity of the pixel, as shown in Fig. 9. This method greatly improves the efficient use of the DAQ board and cancels the noise of the image.

In this system, because there are multiple input channels, the acquisition speed of the PCI-6251 is 1 MS/s. The galvanometer has a maximum scanning speed that is limited by the scan angle. To reach an optimal FOV, the galvanometer should work at ± 1 degree with a maximum scanning speed of ~500 Hz for uni-direction scanning with a filling factor of 66%. When the image is collected at a speed of 500 x 400 at 1 fps, the data collection command is sent at a speed of 800 KS/s. Thus, a 4-point data binning can be performed to match the speed difference between the galvanometer and data acquisition.

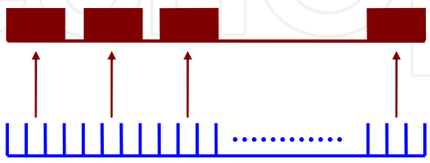
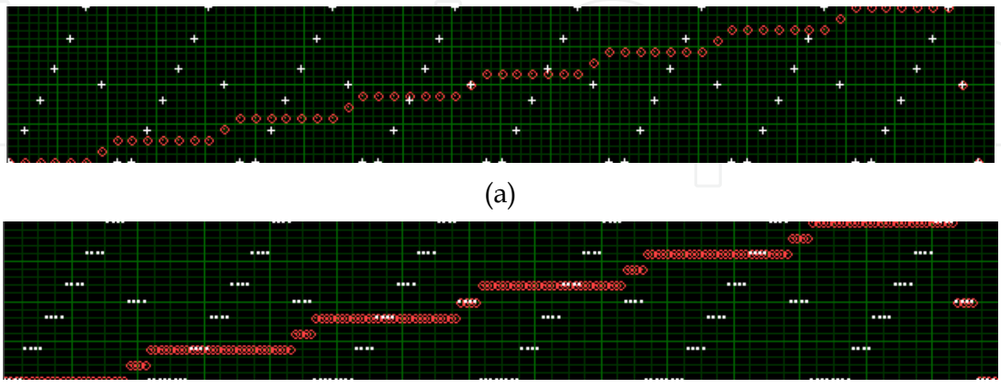


Fig. 9. The data binning algorithm with 4 data points collected and averaged to form one pixel (Chen, Feng et al. 2010).

Compared to the algorithm for frame averaging (Chou, Bower et al. 2005; Nguyen, Tsai et al. 2006), data binning is more robust with respect to sample drift, as each pixel is collected in the neighboring time window. For instance, for the same digitization speed, one can mathematically compare:

1. For frame averaging with an imaging speed of 500 x 500 at 1 fps and an average of four images, the temporal distance between each average pixel is one second, therefore the distortion was averaged one second away from the previous data pixel. The advantage of this mode is that with a sufficient signal (which is usually not the case when frame averaging or data binning is performed), one can observe the drifting movement of the sample from the collected images;
2. For data binning, the same imaging speed requires 2000 x 500 data points in four seconds, and bins every four points. Therefore, the time between each pixel average is four microseconds, a value six orders of magnitude shorter than the frame average.



(a)

(b)

Fig. 10. Comparison of different scan modes: (a) Uni-directional Scan; (b) Uni-directional scan with data binning (Chen, Feng et al. 2010).

2.5 Acquire-on-fly

In CLSM the scan command pixel number and the pixel data collection number are the same (data binning is actually a mathematical method that sacrifices image pixel number for a better SNR). However, due to the fact that the speeds of the galvanometer and data acquisition are different, alternative methods to get around this problem only command several interval spots at one speed (this could be the fastest speed of the galvanometer), whereas data collection runs at another speed (usually much faster than the galvanometric scan for data acquisition techniques currently available). As illustrated in Fig. 11, because the galvanometer's velocity is rather constant when sufficient interval spots are commanded, an image with increased pixel number can be obtained through sequentially remapping the pixels to their respective location (rather than binning the pixels). The conceptual difference between data binning and AOF, relies on the increase of data collection speed in data binning, whereas AOFrelies on a decrease in scanning command speed, relative to the pixel clock.

Fig. 12 illustrates the effect of employing the AOF technique. The application of AOF with a 4 x interval pixel sampling rate between each scan command interval, can improve the image sampling frequency by four times. By adjusting the image aspect ratio accordingly, a square image can be readily collected. Note that in Fig. 12a the horizontal resolution is limited as insufficient pixels are sampled, and the 4x AOF apllied to the image shown in Fig. 12b has effectively increased the resolution. Therefore, for different imaging applications, the user can choose to either acquire a higher SNR image by data binning, or obtain a "larger" image using the AOF technique, depending on the relationships between the SNR, FOV, and Shannon-Nyquist sampling criteria. For example, if the FOV is set to 100 μm x 100 μm, and the optical resolution is 0.5 μm, then to obtain optimal resolution (to meet Shannon-Nyquist sampling criteria) we need at least 400 x 400 pixels. We may use the data binning and AOF together to command the galvanometer to run at 100 x 400 at 1 fps (where the fast axis runs at only 40 K intervals at 400 Hz for a uni-directional scan; the filling factor is

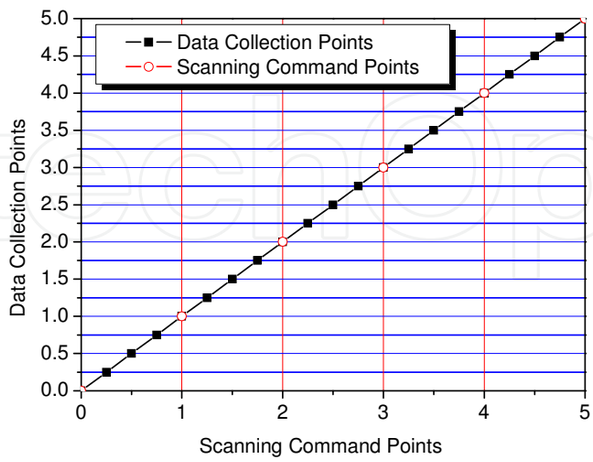


Fig. 11. The mismatch of scanning command speed and data collection speed. The line shows the trace of the galvanometric mirror response. By using data binning and/or Acquire-On-Fly technique, the mismatch can be compensated. (Chen, Feng et al. 2010)

omitted for simplicity) and collect data at 1600 x 400 at 1 fps (where the digitization speed is 640 KS/s). With four times data binning the SNR is improved; and with 4x AOF the image is rescaled to 400 x 400. Of course, the actual resolution is also affected by the SNR of the image, so in practice it is always a trade-off between AOF and data binning, with a fixed digitization speed.

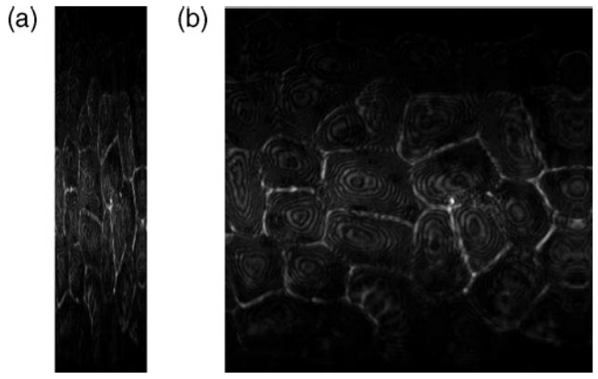


Fig. 12. Confocal images of a bifoliate bush leaf with: (a) conventional synchronized scanning and sampling mode (pixel ratio of X:Y=1:4); (b) Acquire-on-Fly mode with 4x the sampling speed resulting in a square-frame image with 4x the pixel numbers. Image size: 150 μm (Chen, Feng et al. 2010).

3. Real-time imaging with a single spot laser scan

3.1 Fast scan with a resonant scanner

The disadvantage of galvanometric scanning lies in the fact that galvanometer is accurate but slow to scan. To work around this problem, a resonant scanner running at a fixed frequency can be used. To maximize the velocity of scanning, the velocity of the resonant scanner is often a sinusoidal pattern, in which the specimen is scanned at the highest speeds in the central region of the image, where as the velocity progressively decreases as the scan reaches the edges. As a result, when the image data flow derived from a resonant scanner is acquired by a frame grabber clocked at a constant pixel rate (which assumes the beamscans linearly), the images appear stretched at the edges (see Fig. 13).

The key to restoring the distorted image is to generate a proper pixel clock that is syonchronized with the scan velocity. The image can be collected and then post-processed mathematically (Callamaras and Parker 1999). CRS Corp. has developped an electronic pixel clock generation circuit to work with their resonant scanner to provide a pixel clock output corresponding to the scanning velocity (CRS Application Notes 1994). A Ronchi grating can be utilized to optically generate a pixel clock when the beam scans across the grating groove (Leybaert, De Meyer et al. 2005).

3.2 Fast scanning with a polygon mirror

Another way to generate fast angle deflection is to use a rotating polygon mirror. Unlike the resonant scanner, which runs bi-directionally and scans with a sinusoidal velocity, the angle deflection provided by the polygon mirror is uni-directional and temporally uniform. Therefore, for resonant scanning post-processing of the pixel clock is avoided.

However, the disadvantage of a polygon mirror scanner is that spinning velocity is not constant, and fluctuates. A second a split photo-diode is generally used to monitor the velocity fluctuation and generate a corresponding pixel clock (Veilleux, Spencer et al. 2008).

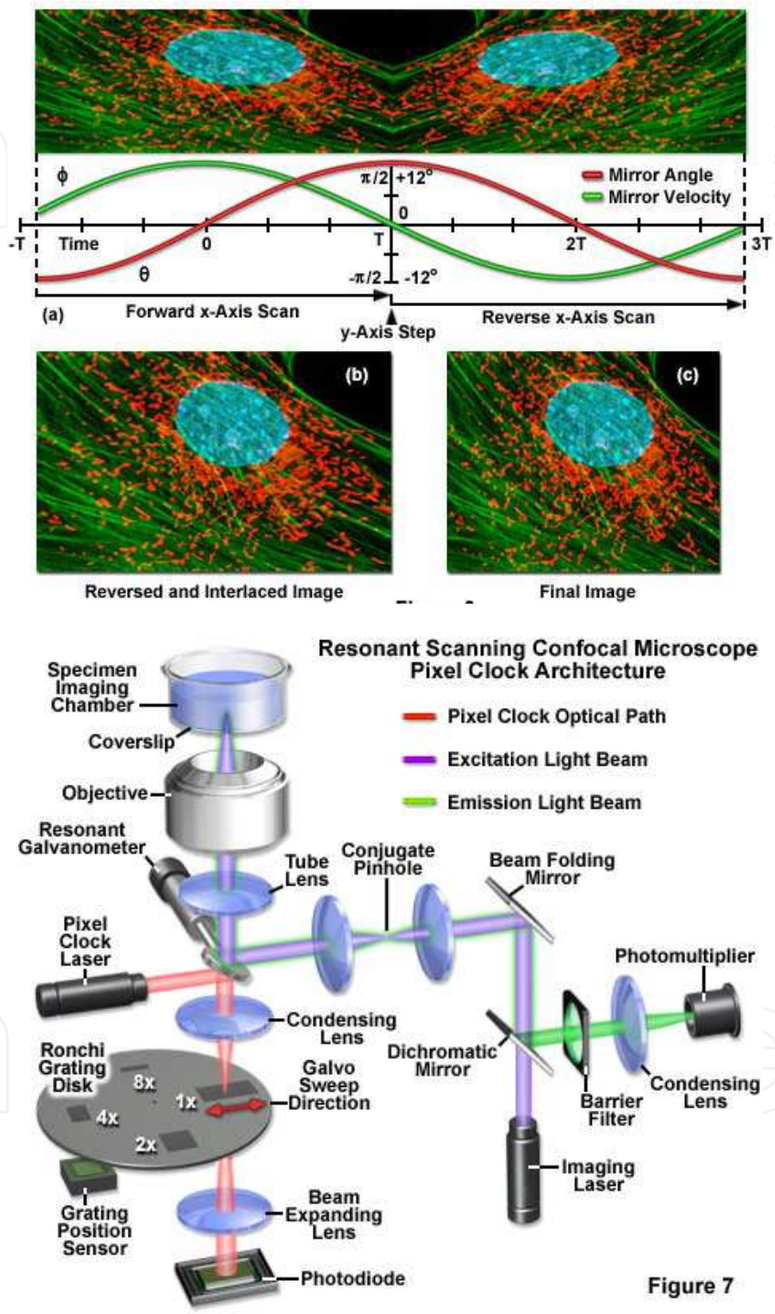
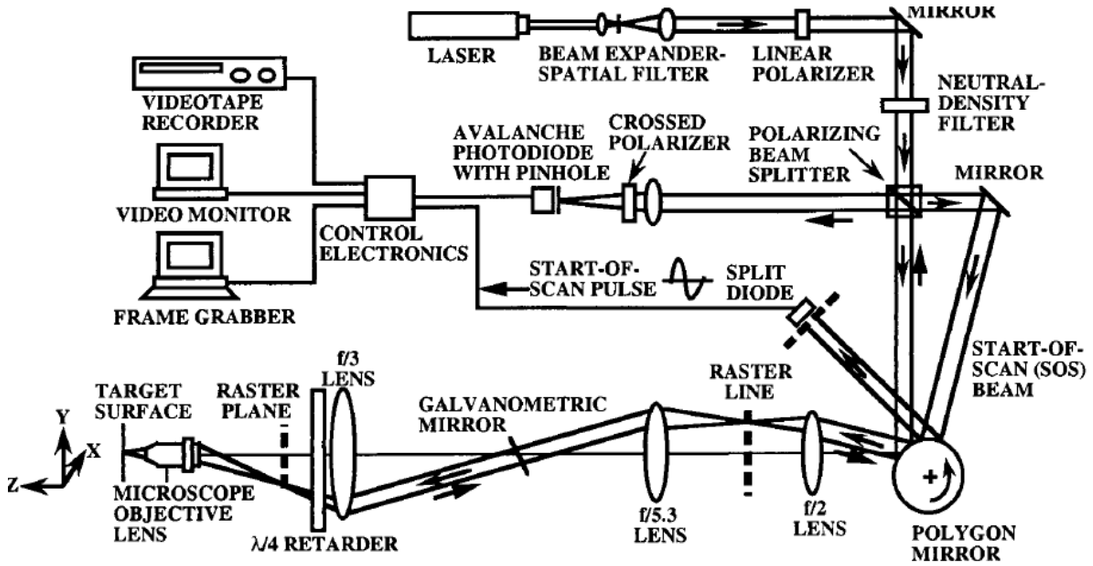
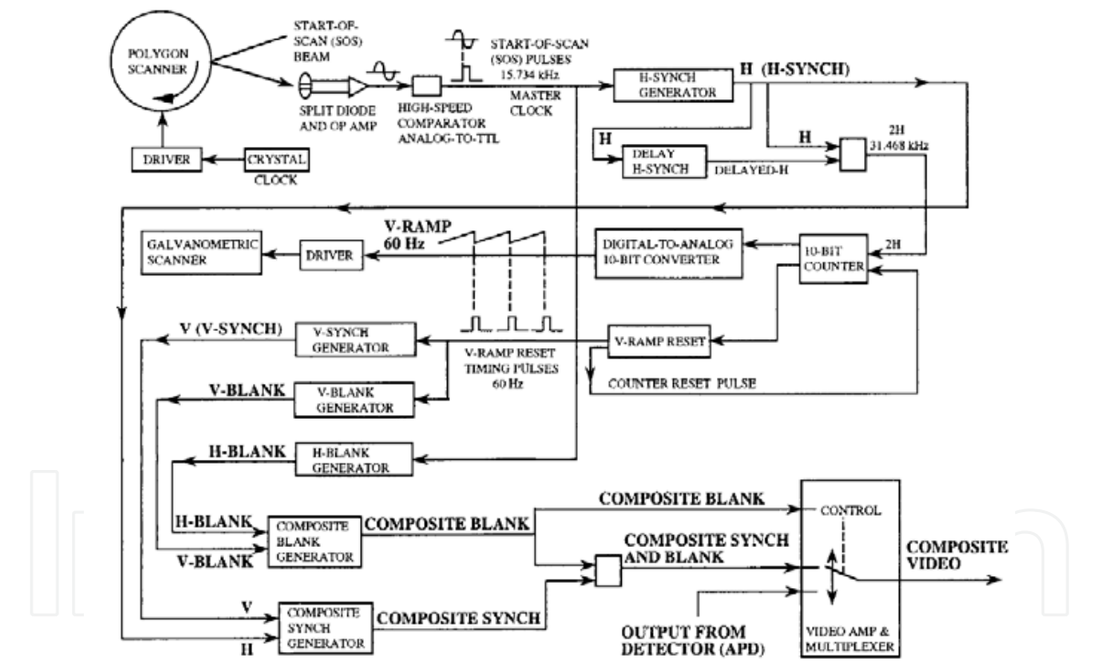


Fig. 13. The resulting distorted and corrected images are shown using the mirror angle and velocity of a sinusoidal resonant scanner; schematic diagram illustrates the optical pixel clock generation architecture
(http://www.microscopyu.com/articles/confocal/resonantscanning.html).



(a)



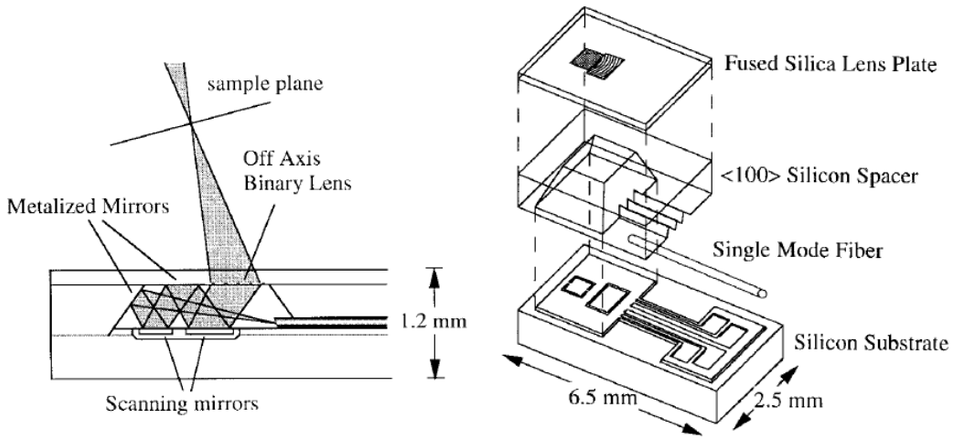
(b)

Fig. 14. (a) Schematic diagram of the polygon mirror based reflective confocal laser scanning microscopy; (b) electronic controlling diagram of the system(Veilleux, Spencer et al. 2008) Image courtesy of IEEE.

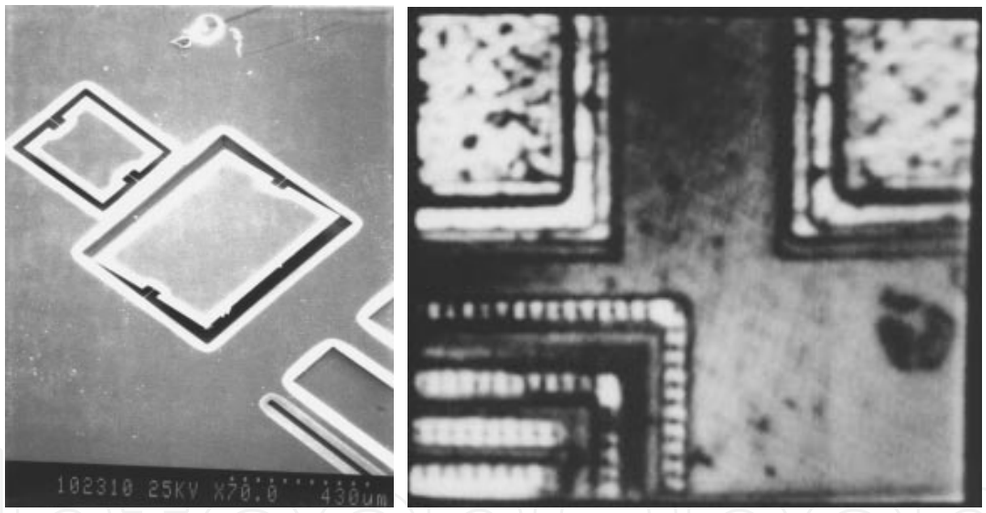
3.3 Endoscopic fast scan with MEMS

Taking advantage of cutting-edge MEMS fabrication techniques, a miniaturazed MEMS scanning mirror can be employed to dramatically decrease the size of conventional scanning

schemes. Dikensheets and Kino (Dickensheets and Kino 1998) reported the instrumentation of a MEMS based confocal laser scaning system, the schematics of which are shown in Fig. 15. The two scanning MEMS mirrors are fabricated from the same silicon substrate.



(a) (b)



(c) (d)

Fig. 15. (a) Schematic diagram of MEMS based CLSM; (b) the diagram of the structure of the MEMS-CLSM system; (c) a SEM image of the MEMS scanning mirrors; and (d) the image result of an IC chip (Dickensheets and Kino 1998) Image courtesy of IEEE.

3.4 Endoscopic fast scanning with fiber vibration

In endoscopy where the size of the probe is limited, optical fiber plays an important role in signal transferring. To obtain a confocal image *in vivo*, a mimiaturized CLSM system for endoscopy can be achieved with fiber vibrating at 2-D (Thong, Olivo et al. 2007). The handheld rigid probe consists of a handle with a focusing knob and an 8 mm diameter metal tube that houses a miniaturized tuning fork and the output end of the single-mode fiber is attached to one of the arms of the tuning fork. A magnetic coil driver induces a 700 Hz

resonant oscillation in the tuning fork, providing a fast x scan, while a second coil causes the entire tuning fork to pivot in the y direction at a slower scanning rate of 1 to 2 Hz. Typically, a 1024 x 512 image can be obtained at 1.6 fps (Goetz, Fottner et al. 2007). The small tip of the single-mode fiber plays acts as both the illuminator and the detection pinhole of the confocal system. The fiber tip can also be driven to scan with a Lissajoul pattern (Helmchen, Fee et al. 2001) or spiral pattern for lateral imaging (Myaing, MacDonald et al. 2006).

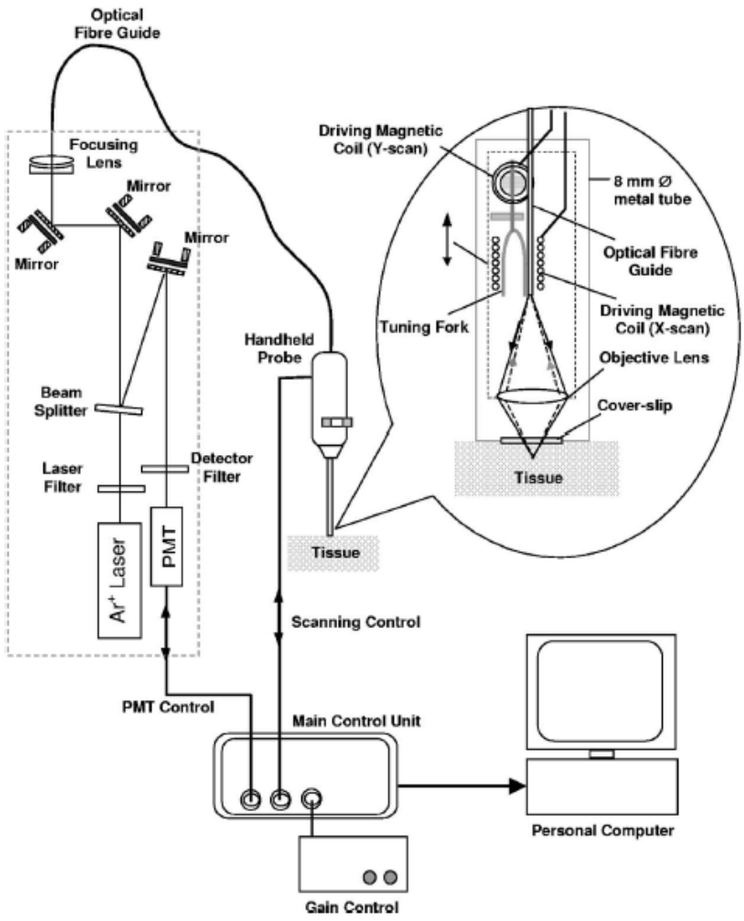
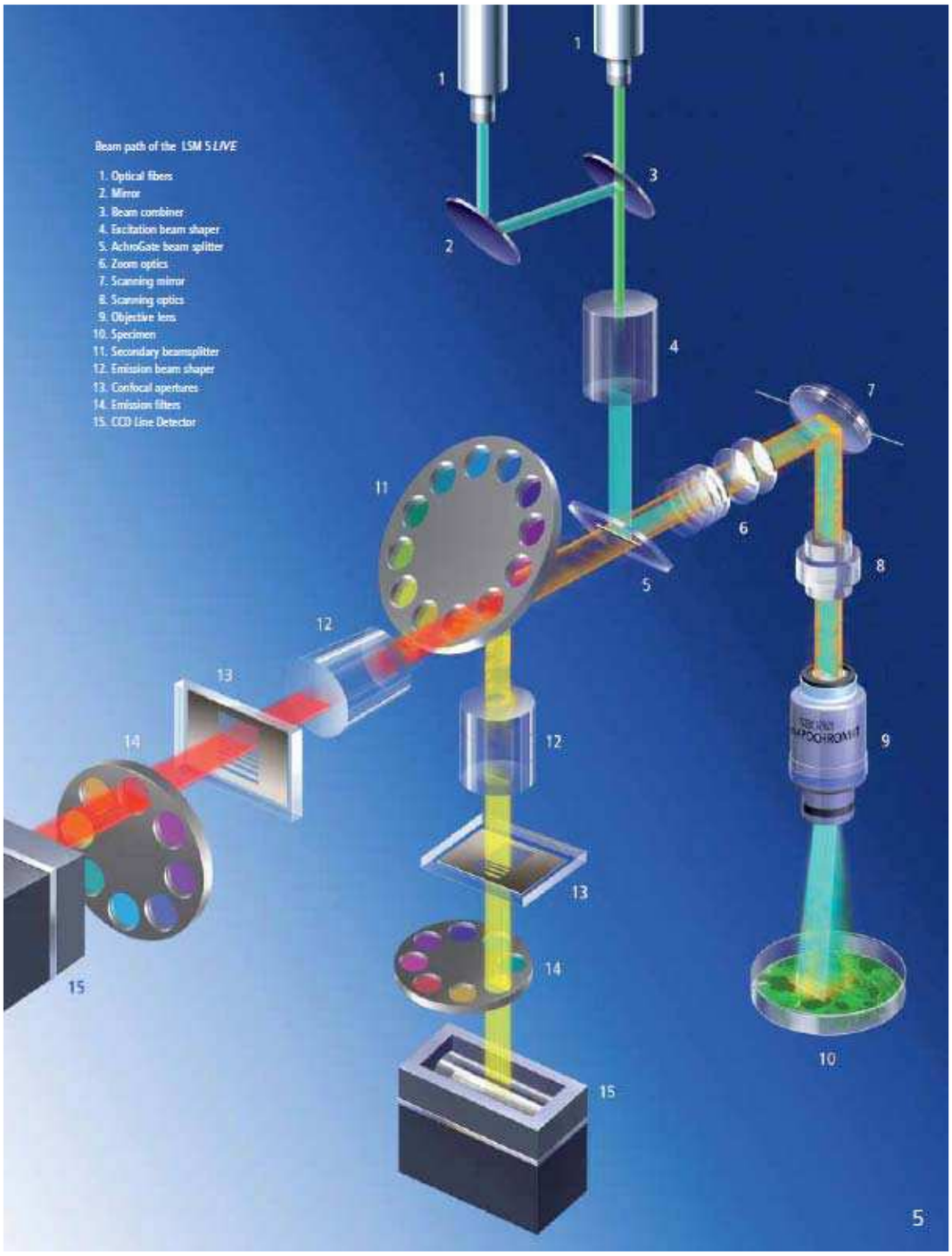


Fig. 16. Diagram of a confocal endomicroscope, which scans using a vibrating optical fiber with two tuning forks (Thong, Olivo et al. 2007) Image courtesy of SPIE.

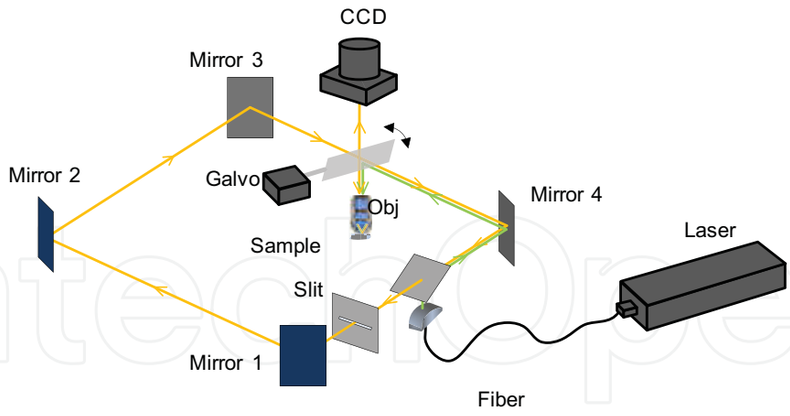
4. Line scanning confocal microscopy

The imaging speed of a single spot scanning system is physically limited by the fast scanning unit. To reach a higher frame rate, one naturally thinks of using parallel spots or line scans. In line scan confocal microscopy, a cylindrical lens or mirror reshapes the light to a line at the focal plane of the objective where the specimen located. Since a galvanometer can reach speeds of ~500 Hz, it is used to scan the line and achieve a high imaging frame rate. The signal from the specimen is de-scanned by the galvanometer and detected by a line detector that is synchronized with the scanning command, thus forming a confocal image. One example of this setup is the Zeiss LSM5 Live system.

However, if such a line signal is re-scanned for 2-D imaging, it can be directly observed by the experimentor, or recorded by a CCD camera. Meridian released such a line scan system in the 1980's (see Fig. 17). In this system, the fluorescence signal from the sample is first de-scanned, then passes through a confocal slit located at the position of the mirror and focal



(a)



(b)

Fig. 17. Schematic diagrams of (a) a Leica LSM Live 5 (image from the manual); (b) a Meridian line scan confocal imaging system.

plane of the cylindrical lens. The back of the galvanometer is then used to re-scan the signal for CCD collection or the image viewed directly by the observer.

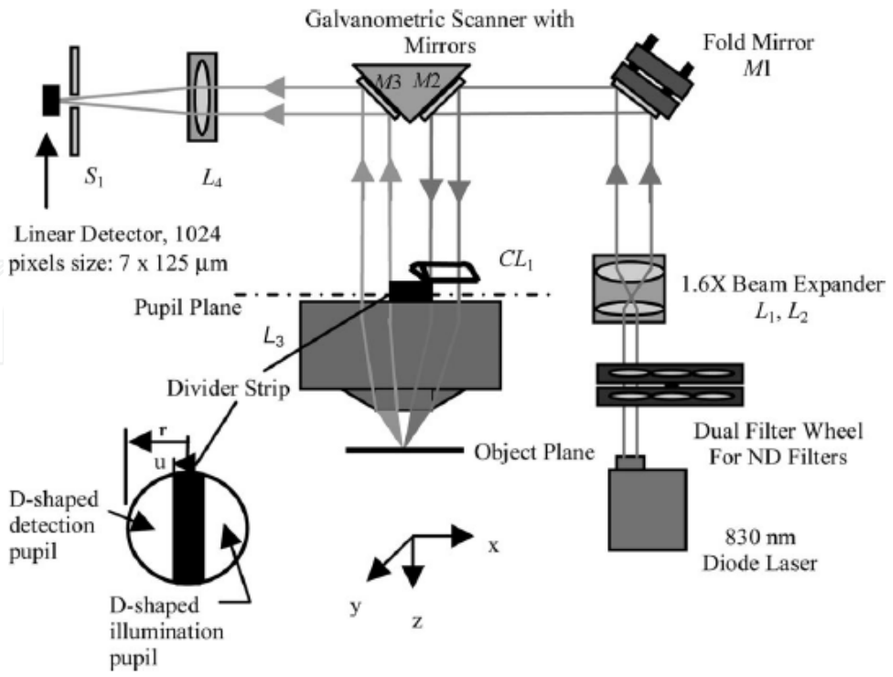
Theta-line scanning is an alternative method to that using in line scanning confocal imaging (Dwyer, DiMarzio et al. 2007). In theta line scanning, a D-shaped detection pupil is used with a f = 200 mm cylindrical lens and a line CMOS camera used for data collection. A non-resonant galvanometric scanner was used to scan across the sample. Similar to the control unit designed by Callamaras and Parker, as outlined in the previous section, the data from the CMOS detector is sent in conjunction with the PCLK, H-SYNC and V-SYNC signals, to a video frame grabber for signal processing. A 1143 x 1024, 4.86 fps image acquisition speed was acheived when using a 1 x 1 mm field-of-view.

5. Simultaneous multiple spot scanning

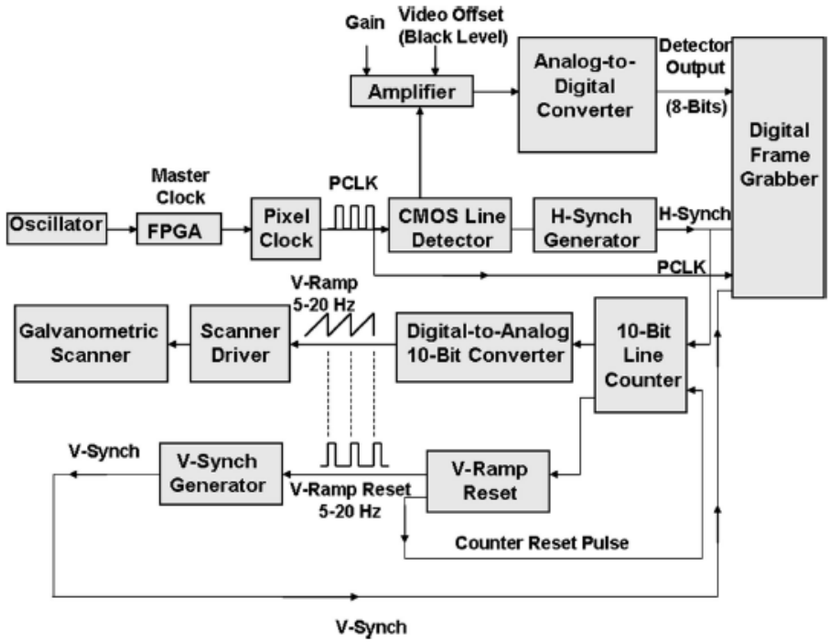
As described in Section 1, the application of a pinhoe in confocal microscopy can effectively block the stray light from the neighbourhood, attaining both improved resolution and the capability to reconstruct sections in 3-D. Multiple pinholes located at distant positions, make it is possible to simultaneously scan the sample with multiple beam foci. The Nipkow disk confocal scanning system utilizes this concept and is illustrated in Fig. 19. The Nipkow disk consists of two concentric disks spinning at the same velocity, and where the top disk is filled with a spinal shaped microlens array and the bottom disk has pinholes that are in correspondence with the lenslets of the upper disk. The illuminated spinning Nipkow disk generates multiple foci on the specimen postioned at the focal plane of the objective and when the disk is spinning fast enough a 2-D CCD detector or the experimentor can then detect the confocal image. One drawback in such a scheme is that the full aperture of the objective is not completely filled by the beam from each lenslet and this results in poor optical section resolution.

6. Conclusion

Confocal microscopy has become a very important tool for biological research as well as clinical diagnosis. Because laser scanning can provide faster imaging speeds compared to



(a)



(b)

Fig. 18. Schematic diagram (a) and control circuit (b) of the theta-line scanning system. A 1024-element CMOS line camera was used for data collection (Dwyer, DiMarzio et al. 2007) Image courtesy of OSA.

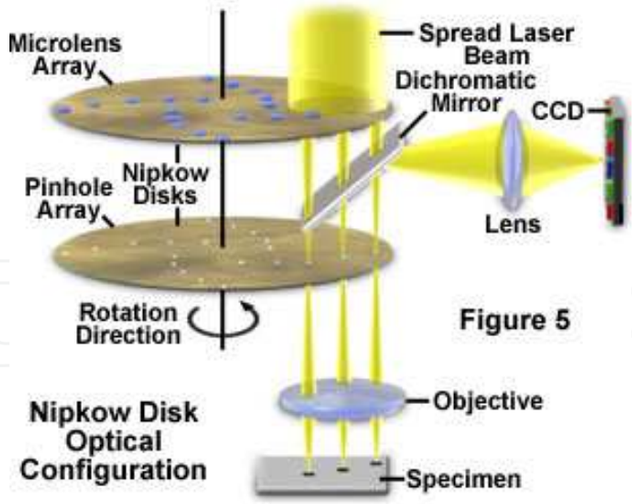


Fig. 19. Schematic diagram of the Nipkow disk scaning system (http://www.microscopyu.com/articles/confocal/confocalintrobasics.html).

conventional stage scanning movements, most confocal microscopic systems employ laser scanning as a means of lateral plane imaging. The most straightforward scanning mechanism can be achieved by using a pair of galvanometers and indeed, such a galvanometric configuration still plays a significant role in commercial CLSM systems. However, the speed of the galvanometer is generally limited to ~500 Hz, which in turn limits the imaging speed of a galvanometric based confocal system to ~1 Hz for a 500 x 500 image.

To obtain faster scanning speeds, a resonant scanner or a rotating polygon mirror can be used to generate a fast scan. Both these methods have their own pros and cons with respect to scanning control and data reconstruction. To minimize the size of the scanning head for confocal endo-microscopy, MEMS techniques can be applied, or through the use of optical fiber vibration. Since the speed of a galvanometric confocal system is limited by the fast line scan, a cylindrical lens can be used to generate a line focus and together with a slit, the confocal image can be detected. The image speed of a flying spot confocal system is limited by to scanning one spot at a time, however, multiple spots can be generated and scanned across the sample with a Nipkow spinning disk confocal system obtain confocal images with 2-D detectors.

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Ever since the invention of laser by Schawlow and Townes in 1958, various innovative ideas of laser-based applications emerge very year. At the same time, scientists and engineers keep on improving laser's power density, size, and cost which patch up the gap between theories and implementations. More importantly, our everyday life is changed and influenced by lasers even though we may not be fully aware of its existence. For example, it is there in cross-continent phone calls, price tag scanning in supermarkets, pointers in the classrooms, printers in the offices, accurate metal cutting in machine shops, etc. In this volume, we focus the recent developments related to laser scanning, a very powerful technique used in features detection and measurement. We invited researchers who do fundamental works in laser scanning theories or apply the principles of laser scanning to tackle problems encountered in medicine, geodesic survey, biology and archaeology. Twenty-eight chapters contributed by authors around the world to constitute this comprehensive book.

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