Yokogawa Spinning Disk

Introduction

There are two significant challenges in biological imaging that conventional fluorescence microscopy cannot overcome. Firstly, biological specimens are 3-dimensional structures so to fully understand them we often need to construct 3-dimensional images. Secondly, many processes biologists would want to study occur inside biological structures, but other cell features such as the cell membrane block a clear view.
These problems are difficult to overcome due to the need to pass light through the entire sample to illuminate the chosen image plane. This gives no control over where within the light path the returning light comes from. Light from out of focus planes, and from bright features above and below the desired plane cannot be blocked with conventional fluorescence microscopy.

Confocal microscopy offers the solution to this issue. Confocal microscopy uses optical sectioning to take multiple, thin, 2-dimensional slices of a sample to construct a 3-dimensional model from them. This process removes the out-of-focus light from other planes.

The first design of a confocal microscope was the confocal laser scanning microscope (CLSM). CLSM uses a laser beam which passes through a single pinhole in the excitation light path, with the emission light from the illuminated point passing through a separate pinhole to a detector, a photomultiplier tube (PMT) (Figure 1).

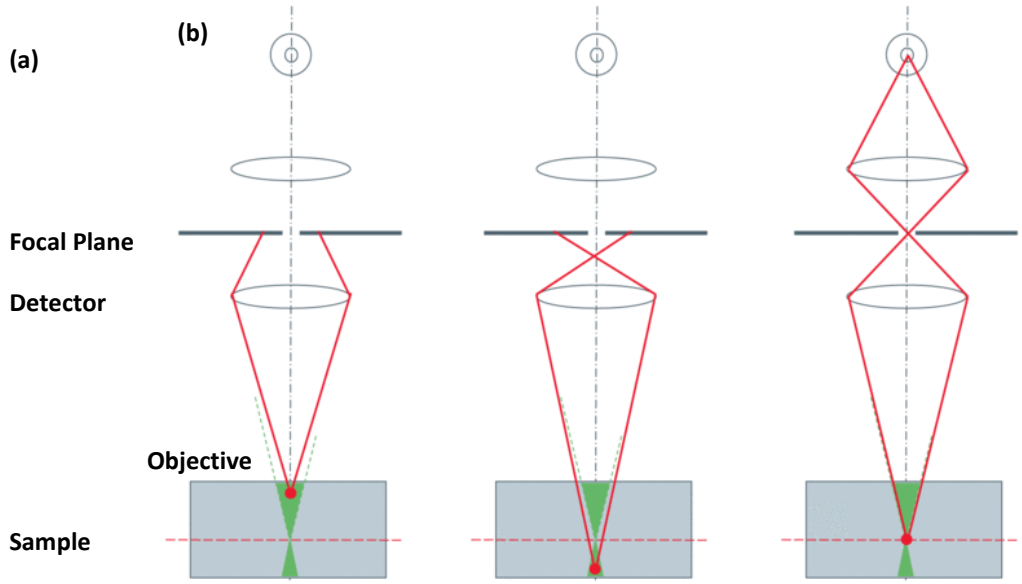

Figure 1: A pinhole aperture in a conjugate image plane blocks out-of-focus light from a) above the sample and b) below the sample. c) Only light from the focal plane passes the pinhole to the detector.
From Leica Science Lab, *http://www.leica-microsystems.com/science-lab/confocal-microscopy/*

To generate an image, this light path is raster-scanned across the sample in 2 dimensions, then an image is reconstructed from the detected light. A piezo Z-stage is used for the z-direction. This technique generates high-quality images but at the cost of very slow speed - on the order of 1 second per image. Additionally, the poor efficiency of the photomultiplier tubes combined with the highly concentrated illumination beam cause considerable photobleaching and photodamage to live cells. Another technique was therefore needed for live cell work, or for fast processes.

Spinning disk confocal microscopy solved this problem by using multiple pinholes to scan the entire image, parallelizing the process and greatly increasing the speed of acquisition. Compared to laser scanning confocal microscopy, spinning disk confocal microscopy is high-speed, high-sensitivity and simple to implement. This makes it a very common choice for studying 3-D structure, fast dynamic processes, long-term time-lapse or details inside the cell membrane, all possible with live cells.

The most advanced design of spinning disk unit was engineered by Yokogawa Electric Corporation of Japan and it remains the 'gold standard' for microscope manufacturers today.

Yokogawa spinning disk principle

The Yokogawa spinning disk unit consists of two coaxially aligned disks, a collector disk, and a pinhole disk, with a dichroic mirror positioned between them. Each disk contains pinholes arranged in a series of nested spirals. The pinholes on the collector disk contain Fresnel microlenses that focus light onto pinholes laterally co-aligned on the pinhole disk where the pinholes are at the focal plane of the microlenses (Figure 2).

Laser light is introduced from above and projected onto the collector disk through a collimating lens. The light passes through the microlenses and the dichroic mirror and onto an area of pinholes on the pinhole disk. When the disks are spun, the pinholes scan across entire image rows in sequence. The holes are positioned so that every part of the image is scanned as the disk is turned. Each area of the image is scanned by a single pinhole (typically) every 30° rotation of the disk. The rotation speed of the disk, therefore, determines the maximum image acquisition speed.

Fluorescence emission from the sample is collected by the objective lens and focused

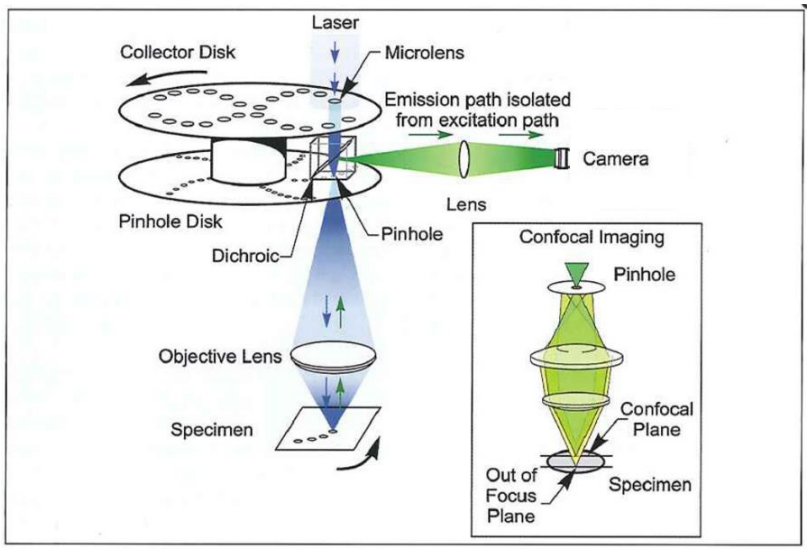

Figure 2: Schematic showing the principle and optical components of a Yokogawa spinning disk confocal microscope. The collector disk contains a pattern of microlenses which are aligned with the pinhole patterns. The disks are fixed to an electric motor shaft where they are separated by a distance equal to the focal length of the microlenses. Spinning the disks causes an array of focused laser beams to be scanned across the specimen. The fraction of the in-focus fluorescence emission light which returns along the illumination path is preferentially passed to the pinhole array and reflected into the camera port by the dichroic mirror located between the two disks (Nelson *et al.* 2010)

back onto the pinhole disk. The pinhole blocks out-of-focus light from passing through the disk so illumination light from one pinhole creates emission light that's gathered by the same pinhole. This light is reflected by the dichroic mirror and passes through an emission filter before being focused onto the camera sensor for detection.

Spinning disk microscopy is, essentially, a light rejection technique so a high photon budget is necessary for high-quality imaging. For this reason, the Yokogawa spinning disk unit uses beam shaping, collimating lenses and microlenses to substantially increase the amount of light throughput to increase photon collection efficiency. Furthermore, the pinhole pattern ensures that image frames are homogenously illuminated so there are no light intensity gradients across the field of view and no scanning stripes.

Types of Yokogawa spinning disk

There are currently two main types of Yokogawa spinning disk unit; the CSU-X1 and the CSU-W1. The mode of operation is the same but there are important differences between the two.

The CSU-X1 is the faster unit with scanning speeds of up to 10,000 rpm and a theoretical maximum acquisition speed of 2000 fps.
The CSU-W1 has a slower scanning speed of up to 4000 rpm and a theoretical maximum acquisition speed of 200 fps but the field of view is almost 4 times larger to better accommodate larger field of view camera sensors such as new state of the art CMOS devices. It also features decreased crosstalk and an extended near-infrared spectral range, allowing for sharper images of regions deeper inside live cells.

Historically, there were two other unit models, the CSU-10 and the CSU-22 which differed by having rotational speeds of 1800 rpm and 5000 rpm with maximum frame rates of 260 fps and 1000 fps, respectively. These unit models are no longer being produced but there remain a significant amount of them still in use.

When performing fast imaging with Yokogawa spinning disk units, the disk rotation speed must be adjusted to match camera exposure time. As mentioned before, each area of the image is scanned by a single pinhole every 30° rotation of the disk, therefore the exposure time of the camera must be an integral multiple of the time necessary to perform one 30° rotation. If exposure times are not matched to the scan speed, striped patterns can become superimposed on the image. This is not so important when using long exposure times (~100 ms) but will become a problem if it's necessary to image at 200 fps (5 ms exposure time) and above.

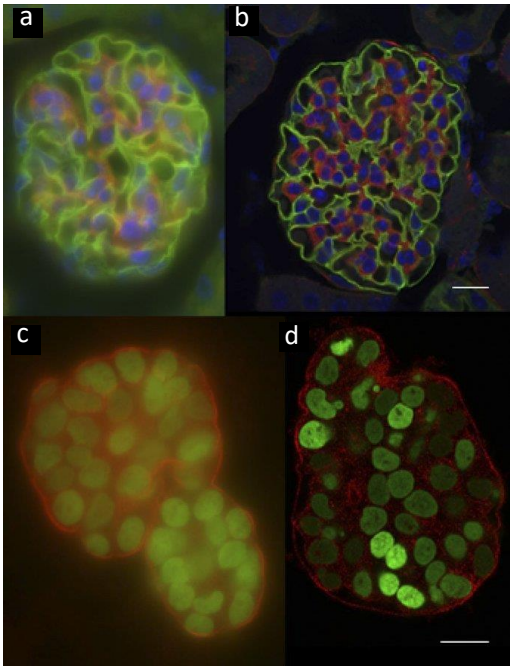

Figure 3: (a) Widefield image and (b) confocal image of ~20 µm thick mouse kidney section. (c) and (d) show widefield and confocal images of ~50µm thick mammary epithelial cells. Scale bars 20µm. Adapted from **(Jonkman and Brown, 2015)**

A second camera port is commonly used with Yokogawa spinning disk units to allow simultaneous two-color imaging or, historically, to have a high sensitivity EMCCD on one port for low-signal imaging and a CMOS camera on the other port for larger field of view, higher speed imaging. However, with the advent of high sensitivity, back-illuminated CMOS devices, these two functions can now be performed with one camera.

Yokogawa spinning disk camera choice

Any camera can be used with a Yokogawa spinning disk unit but the best performance can be achieved with a sensitive camera with high speed and a large field of view.

Spinning disk microscopy is, by definition, a light rejection technique so maximizing the number of photons detected by the camera is of high importance. This has historically been achieved with EMCCD cameras which are highly sensitive but have a relatively small field of view and slow speed.

More recently, sCMOS cameras have been used for spinning disk confocal microscopy due to their large field of view, high speed and varied pixel sizes to maximize resolution - a pixel size of 6.5 um will ensure Nyquist sampling with 60x magnification and a pixel size of 11 um will ensure Nyquist sampling with 100x magnification. However, front-illuminated sCMOS devices do not match the sensitivity of an EMCCD which means that longer exposure times and a higher light dose need to be used which has negative consequences for the sample and prevents high speed from being reached.

Even more recently, a more attractive option has been back-illuminated sCMOS cameras which have equivalent sensitivity to an EMCCD camera with the field of view, speed and pixel size advantages of sCMOS technology. This allows sensitivity to be maximized without having to trade-off speed or field of view, ensuring faster, more physiologically relevant data acquisition.

References

Jonkman, J. and Brown, C. M. (2015) Any Way You Slice It - A Comparison of Confocal Microscopy Techniques. *Journal of Biomolecular Techniques* 26: 54-65.

Nelson, M., Ledoux, J., Taylor, M., Bonev, A., Hannah, R., Solodushko, V., Shui, B., Tallini, Y., & Kotlikoff, M. (2010) Spinning Disk Confocal Microscopy of Calcium Signalling in Blood Vessel Walls. *Microsc Anal* (Am Ed). Mar 1; 24(2): 5–8.

https://www.yokogawa.com/eu/

