Spinning disk confocal microscopy

spinning disk confocal scanner head



Britta Schroth-Diez



Zeiss Axioimager, Yokogawa CSU-10 spinning disk scan head, CCD camera





mammalian cell, Rab5-GFP

Claudio Collinet, Zerial lab

1 frame – 0.3 sec, playback 15 frames/sec



Paul Nipkow, 1860 – 1940, Germany



Der hier zu beschreibende Apparat hat den | Der hier zu beschreibende Apparat hat den Zweck, ein am Orte A befindliches Object an einem beliebigen anderen Orte B sichtbar zu elektrischen Stromes zu drehen a B ein Cu



http://inventors.about.com





Obr. 2

United States Patent Office

3,517,980

Patented June 30, 1970

1

3,517,980 METHOD AND ARRANGEMENT FOR IMPROVING THE RESOLVING POWER AND CONTRAST Moimir Petran and Milan Hadravsky, Plzen, Czechoslovakia, assignors to Ceskoslovenska akademie ved, 5 Prague, Czechoslovakia Filed Dec. 4, 1967, Ser. No. 687,638 Claims priority, application Czechoslovakia, Dec. 5. 1966, 7.720/66, 7.721/66 Int. Cl. G02b 21/06, 17/00, 27/02 5 Claims ¹⁰

U.S. Cl. 350-17

ABSTRACT OF THE DISCLOSURE

The observed object is illuminated by light spots scan- 15 ning subsequently the whole area of the observed object, and only a part of the light coming from the observed object due to illumination by said light spots of an area which corresponds to the area of the original light spots is allowed to enter the image plane of the microscope ob- 20 jective. The resolving power is thus substantially improved and observation of objects covered by translucent material such as living cells and nerves below the skin is enabled.

2

observed object point. The light passing through each filter impinges on its own photocell and the subtraction is accomplished electrically. The resulting signal is again transformed to light and the image is reconstructed by scanning rotating mirrors.

It can be assumed that the realization of the method based on this principle is rather difficult if not impossible as the demands for exact localization of images on filters, transposed to accuracy of angles and the synchronization of rotating mirrors and the accuracy of their axle bearings reach small fractions of seconds and several manometers. Requirements for stability and linearity of amplifiers are similarly rather high. For a suitable scanning density and/or suitable size of the field of view, the result is either an enormous rotation speed of the mirrors or a rather long time interval necessary for a complete image to be formed.

Stray light is another cause of the redaction of resolving power and of the contrast of images. Sources of stray light can be reflection on optical surfaces of the microscope components and diffraction in different layers of a thicker object observed. This stray light particularly deteriorates images in incident light microscopes.

The stray light caused by the reflection on optic sur-



Mojmir Petran, Milan Hadravsky, Pilsen, Czech Republic



en.wikipedia.org/wiki/Iron_Curtain



Spinning disk confocal microscopy

- "field" illumination method multipoint scanner
- detector : CCD, EM-CCD, sCMOS
- temporal resolution typically in a range of dozens to hunders of ms/image for "standard" FOV: 512x512 pixels
- lower Z resolution compared to LSCM
- circa 800 nm as Z resolution at best
- more out of focus signal

What is it good for ?

- high speed live cell imaging
- fast imaging of fixed samples