Live Cell Spinning Disk Confocal Fluorescence Imaging of Cells on a Zeiss Axiovert Microscope
AfCS Procedure Protocol PP00000135
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The following procedure describes the acquisition of confocal fluorescent and bright field images of live cells, expressing cyan fluorescent protein (CFP) and/or yellow fluorescent protein (YFP), with a spinning disk confocal head on a Zeiss Axiovert 200 M microscope.

Description of Microscope and Imaging Setup
1. Live cells are prepared on glass coverslips for this procedure.
2. The microscope is an automated Zeiss Axiovert 200 M with a 1.4 NA 100x oil objective.
3. A Yokagawa confocal spinning disk containing a dual laser transmitting dichroic is attached to the left camera port of the microscope.
4. Helium-cadmium and argon-ion lasers are focused onto two respective laser-to-fiber couplers and then onto the respective ports of a Y-shaped optical fiber that aligns the laser beams onto a 460 long-pass dichroic. The combined laser beams are brought into the confocal head through the third port of the combining fiber.
5. Excitation and emission wavelengths are controlled with filter wheels attached to the Yokagawa spinning disk head and controlled with a lambda-10-2 filter wheel controller. This modification of the Yokagawa head is available from PerkinElmer.
6. A 442/10 or 515/15 nm band pass filter is used for exciting CFP or YFP, respectively.
7. Laser power is measured at objective port with an optical power meter. For CFP excitation, at 442 nm, laser power is typically between 550 and 600 µWatts. For YFP, the laser power supply is adjusted to set the 514 nm light to between 190 and 210 µWatts.
8. A cooled charge-coupled-device (CCD) camera is attached to the Yokagawa head for image acquisition.
9. A 490/50 or 565/75 nm band pass emission filter is used for CFP or YFP, respectively.
10. Hardware control and image acquisition are performed with Metamorph software.

Description of Acquisition Parameters
1. Twelve-bit images (intensity values of 0 to 4095) are acquired with the camera binning set to 2 x 2. This, combined with the 100x objective and 6.5 x 6.5 µm CCD chip pixel dimensions, results in images that have pixel dimensions of 0.13 x 0.13 µm.
2. When acquiring movies, camera exposure time is set to 500 or 300 msec for CFP or YFP, respectively.
3. When acquiring static images, camera exposure time is set to 2000 msec for both CFP and YFP images.
4. Bright field images are acquired with the halogen lamp set to 4 volts and a camera exposure time of 500 msec.
Image Processing
1. A series of background images (20) of a chamber with appropriate buffer but without cells is acquired on each day of experimentation for both CFP and YFP imaging. The images are averaged to create an average background image.
2. The average background is subtracted from each plane of the experimental image.
3. Images are cropped to generate a panel that is 300 pixels or 38.4 µm on each side.
4. The 12-bit images are scaled between 0 and the highest intensity prior to converting to 8-bit images (0 to 255) for display.
5. The original 12-bit intensity scale is shown on the bottom left of each image.
6. Additional information such as the construct name and the scale bar are stamped on the images.
7. Montages of the cropped images are generated to view different planes of the expressing cell simultaneously.

Movie Processing
1. Background values are subtracted from each plane of a movie as described above.
2. The bleach rate of the fluorescence is calculated by measuring the progressive decrease in intensity in the series of images.
3. Movies are corrected for bleaching by normalizing each image in the series for the estimated loss of fluorescence at its respective exposure time.
4. Images are cropped to generate a panel that is about 38 µm on each side.
5. The 12-bit images are scaled between 0 and the highest intensity of all the planes that are incorporated into the movie, prior to converting to 8-bit images (0 to 255) for display.
6. The original 12-bit intensity scale is shown on the bottom left of each image.
7. Movies are saved as AVI files that will play back at 10 frames per second. Compression is done with the Cinepak Codec by Radius.

Reagents and Materials
Axiovert 200 motorized microscope stand: Carl Zeiss Inc.; catalog no. 0000001005820

Zeiss immersol 518F objective oil: VWR International; catalog no. 41800-488

Plan-Apo NA 1.4 100x oil objective: Carl Zeiss Inc.; catalog no. 440782

Yokagawa spinning disk head with excitation and emission filter wheels, model CSU10 UltraVIEW live cell imager confocal scanhead: PerkinElmer; catalog no. 611005

CFP/YFP dual dichroic in Yokagawa head: Chroma Technology Corp.; 442-514T PC
Helium-cadmium laser, 100 mW: Kimmon Electric Co.; catalog no. IK4101R-F

Helium-cadmium laser power supply (117VAC): Kimmon Electric Co.; catalog no. DR1601C-F

Argon-ion laser, 300 mW: Melles Griot; catalog no. 35LAL030-220

Dichroic in fiber: Chroma Technology Corp.; catalog no. 460DCLP

Laser-to-fiber coupler (514 nm): Oz Optics Ltd; part no. HPUC-23AF-514-S-3.9AS-2

Laser-to-fiber coupler (442 nm): Oz Optics Ltd; part no. HPUC-23AD-442-S-6.2AS-11

Laser fiber combiner: OZ Optics Ltd.; part no. WDM-12P-111-442/514-3.5/125-SSS-40-3AF3AF3S-3-3-SP

CFP excitation filter: Chroma Technology Corp.; catalog no. D442/10x

YFP excitation filter: Chroma Technology Corp.; catalog no. D515/10x

Optical power meter and sensor: Advantest; catalog nos. TQ8210 and Q82017A, respectively

Cooled CCD camera, Photometrics CoolSNAP HQ: Roper Scientific; catalog no. CoolSNAP HQ

CFP emission filter: Chroma Technology Corp.; catalog no. HQ490/50m

YFP emission filter: Chroma Technology Corp.; catalog no. HQ565/75m

Image acquisition and analysis software, the latest version of Metamorph: Universal Imaging Corp.; catalog no. 31290

1 GHz Pentium III computer manufactured by Omni Tech Corporation with 1 GigaByte of RAM: Universal Imaging Corp.; catalog no. OT1010

Cinepak Codec: Radius; The Cinepak Codec is packaged with Apple QuickTime Player and comes packaged with freely available movie players from a variety of other sources, including Cinepak http://www.cinepak.com, or the codec can be downloaded separately from Microsoft http://www.microsoft.com/downloads.

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