Photonic Science

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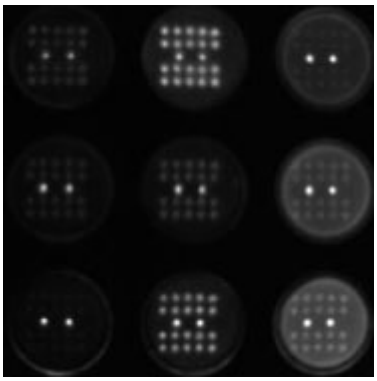
COOLVIEW FDI CAMERA

The Western Blot (alternately, immunoblot) / Northern Blot Gel Imaging is used for detecting specific proteins.

This uses gel electrophoresis order to separate proteins according to their molecular weight and conformal state. The proteins are then transferred to a membrane where they are detected using antibodies to the target protein.

Good dynamic range is required for sensing both bright and low intensities in the same image; typically a 16 bit image digitisation allows good contrast resolution. Reasonable spatial resolution, typically 1.4 megapixel is necessary in order to allow image segmentation for quantitative analysis. Alternatively, a variant technique with a chemiluminescent agent can be used for producing luminescence in proportion to the amount of protein.

A high sensitivity camera is then used to record an image of the antibodies bound to the blot. Usually longer exposures up to minutes are necessary hence a very low dark current / read out noise must be achieved on the camera.



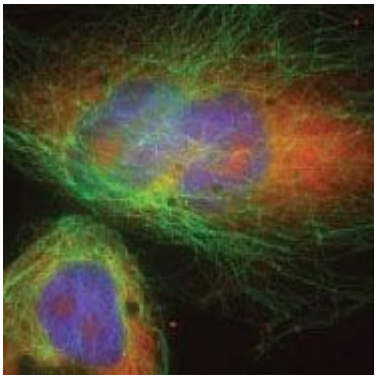
Western Blots / Northern Blot Gel Imaging

Confocal Fluorescence Imaging.

In a confocal microscope, the detector aperture obstructs the light that is not coming from the focal point. The out-of-focus light is blocked by the pinhole and in order to produce crisp image with haze contribution introduced by the depth of field from the objective. The smaller the pinhole, the sharpest the image will be as it will block effectively the fluorescence from the nearest neighbouring planes, but also the less light will be caught by the detector. Therefore there is a requirement for a very sensitive camera in order to capture the fluorescence arising from the confocal plane.

Typical integration time must be kept as short as possible in order to avoid cell damages under extended periods of digital recording. Typically 100ms to 1 second is used per image, depending on sensitivity settings chosen on the camera.

Three dimensional re construction is achieved by acquiring multiple images at different Z positions using a piezo / stepper motor driven z axis on a motorized microscope. Z stacks acquired over time allows to build multidimensional data sets taking into account multiples variables such as wavelengths, x,y,z positions and time. Multiple cameras can be synchronized as one single detector in order to produce parallel acquisition.



Confocal Fluorescence Imaging

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TIRF

A Total Internal Reflection Fluorescence Microscope uses evanescent waves to selectively illuminate and excite fluorophores in a restricted region of the sample. Evanescent waves are generated only when the incident light is totally reflected. The evanescent electromagnetic field decays exponentially and thus penetrates to a depth of only approximately 100 nm into the sample.

Thus the TIRFM enables a selective visualization of surface regions such as plasma membrane. TIRF can also be used to observe the fluorescence of a single molecule. Large magnification objectives are used routinely: typically 63 up to 100x objectives are combined with x2 projective optics in order to reach less than 100nm resolution.

As the volume probed is very small, the amount of fluorescence recorded is small and hence requires very high sensitivity cameras. Exposure time from 10ms to less than 1sec are also necessary in order to record live data from biological samples.

TIRF