FISH

An overview of Andor's solutions for FISH

FISH has evolved into a core technique spanning many areas of diagnosis and research, including cancer cytogenetics, prenatal screening of chromosomal aberrations, molecular pathology and developmental molecular biology. The general principle of FISH, hybridizing fluorescently-labelled loci-specific, telomeric or centromeric DNA probes to chromosomes, can be applied to a range of specimens such as metaphase chromosome spreads or to interphase nuclei and cells. The incentive for multiple labeling of chromosomes and subsequent detection in multiple colors has given rise to Multi-color FISH (M-FISH), fuelled by the desire to maximize the efficiency of the FISH process when applied to situations such as the study of the complex translocations often found in cancer cytogenetics. It is possible to detect multiple targets with fewer fluorophores (e.g. 3 fluors for 7 targets) based on labeling the probes combinatorially, with more than one fluor in various ratios. To simultaneously discriminate all 24 human chromosomes, 5 fluorochromes are required, each fluorescing in a distinct spectral region.Complex "acquired" chromosome abnormailities are common in cancer cytogenetics, ranging from amplification of chromosome number to various defined translocations. One technique which is particularly suited to study of tumour DNA is Comparative Genomic Hybridisation (CGH), a development of chromosome painting, which detects a deviation of the ratio of two fluorochromes to indicate amplification or deletion along the chromosome strand. FISH can also be instrumental for gene mapping. Genes and DNA segments are continually being assigned to chromosome bands, but it is often necessary to determine the precise arrangement of each sequence relative to another at a more detailed level.

From an instrumental standpoint, many adaptations of the FISH technique share a common need for high resolution, sharp contrast between signal and background (particularly in interphase cytogenics), and high sensitivity for achieving high signal to noise ratio from weak signals, or for determining minor comparative differences in CGH. This gives rise to the need for flexible scientific cameras, capable of operating effectively under both "standard" and "ultra-senstitive" modes should the need arise. Furthermore, when performing M-FISH it is desirable to limit the cycle through a range of filter sets corresponding to the number of fluorophores markers being used. High sensitivity operation means that shorter exposures can be used for each filter setting, improving the overall throughout of the technique.It can also be highly desirable to reduce the amount of out of focus haze that restricts both detection limits and contrast of the imaged specimen. This can be addressed in two key ways, either through application of deconvolution algorithms that act of a z-series, or by physically restricting the passage of out of focus fluorescence by insertion of a confocal scanning head - both solutions can be provided by Andor. The ability to scan in the z-dimension also opens the way to three-dimensional FISH (3D-FISH), particularly applicable to interphase cytogenetics.

Andor's EMCCD is a novel detector technology for incorporation into your FISH set-up. EMCCD cameras use an on-chip amplification technology that can be accessed to amplify the signal above the read noise floor. In light-staved instances, one simply needs to apply some EM gain to amplify the previously undetectable signal into a respectable marker. The real beauty of the EMCCD is in its flexibility to operate as a standard high Quantum Efficiency (QE) CCD, or as a single photon sensitive power-horse when the signal is weak and/or exposure times need to be reduced. One can also preserve labelled FISH specimens from the effects of photo-bleaching by filtering the power of the illumination light and compensating with higher EM-gain.