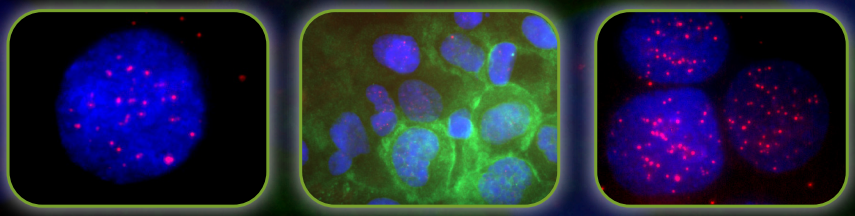
X-Cite®
Fluorescence Illumination • In Control

# Fluorescence In-Situ Hybridization Microscopy

## Application Overview



Fluorescence In-Situ Hybridization (FISH) is a molecular cytogenetic technique that uses fluorescent probes to label nucleotides (DNA and RNA). It allows the detection localization of a specific DNA sequence or genes on an entire chromosome, providing a way to map and visualize the genetic material in a cell and providing spatio-temporal information about gene expression within cells and tissues.

Using multiple probes labeled with a mixture of different fluorescent dyes, each sequence or gene can be labelled in its own unique color. The fluorescent probe binds to only particular parts of a genetic sequence thus offering a very high degree of specificity.

FISH is utilized in diagnosing genetic diseases, gene mapping, and identification of chromosomal abnormalities and may also be used to study comparisons among the chromosomes' arrangements of genes in normal vs. disease states.

Fluorescence in-situ hybridization microscopy has traditionally relied on mercury lamps for the high-power illumination required to image subcellular fluorescence. With advancements in LED technology, there are now mercury-free LED solutions available to address this power-hungry application.

## The Challenge

Since FISH targets RNA or DNA, something much smaller than proteins, the fluorescence signal can sometimes be very low, depending on the protocol employed for staining the sample. As there are multiple fluorophores to be imaged in FISH imaging, narrow-band filters are often used to enable signal separation, which also has the less-desirable effect of reducing the signal intensity. Thus, between potentially low signals and narrow-band filter use, a powerful illumination source is required to excite fluorescence in these samples. This is why many FISH imaging labs continue to rely on mercury arc lamps, even though LEDs have become a more common excitation source for other imaging applications.

## Case Study

Telomeres are structures at the ends of chromosomes that consist of sequential (TTAGGG) non-coding DNA repeats and associated proteins. Normal cells stop dividing and enter senescence after their telomeres reach a critical length. By contrast, cancer cells continue their unlimited proliferation. Immortalization of cells in some cancers has been linked to abnormal activity of telomerase and/or abnormal telomere lengthening patterns. Studies show that altered 3D organization of telomeres in the nuclear space of cancer cells have been associated with genomic instability and correlated with disease progression and aggressiveness.

## Methods

Human cancer cells underwent FISH with cyanine 3 (Cy3)-labeled peptide nucleic acid (PNA) probe to label the telomeres. Cell nucleus was stained by DAPI and CD30 was stained with Alexa488. Three-dimensional images of the cells were acquired using a Zeiss Axio Imager.Z2, equipped with an AxioCam HRm camera and X-Cite XYLIS™ II, using a 63×/1.4 oil plan apochromat objective lens. The telomeres appear as red signals (dots).

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## Results

The FISH images were used for developing a test that would allow predicting the aggressiveness of certain cancers and potential response to treatment based on the 3D distribution and intensity of the telomeres. Since it is imperative to maintain the same intensity of the light source to be able to accurately quantify the required parameters, the output of the XYLIS II had to match the previously used lamphouse. Optical power and hence exposure times for the XYLIS II vs. the old lamphouse were similar. Compared to the lamp system, the XYLIS II has no warmup time, and the intensity on the XYLIS II can be preset and remembered.

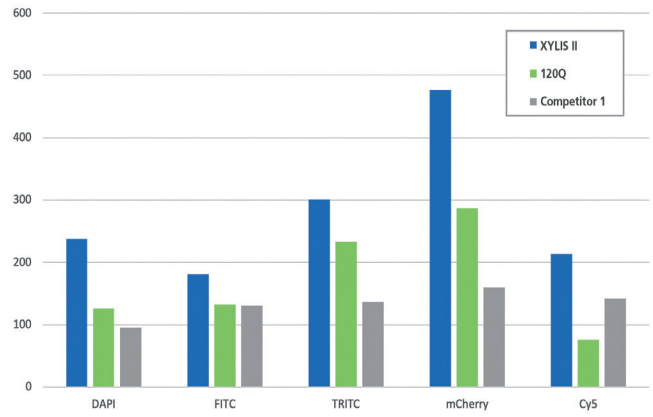
## The Solution

X-Cite XYLIS II with LaserLED Hybrid Drive® Technology: The unit uses laser phosphor conversion to fill in the challenging "Green Gap" (540-590 nm) where powerful LED excitation light was a successful replacement for outdated lamphouse technology in this busy FISH lab. In addition, the XYLIS II features quiet operation for busy FISH lab stations and has boosted power in the following wavelengths: 385, 430, 460, 635 and 730 nm to excite the wide range of fluorophores used in this application.

### Why Use X-Cite XYLIS II for FISH?

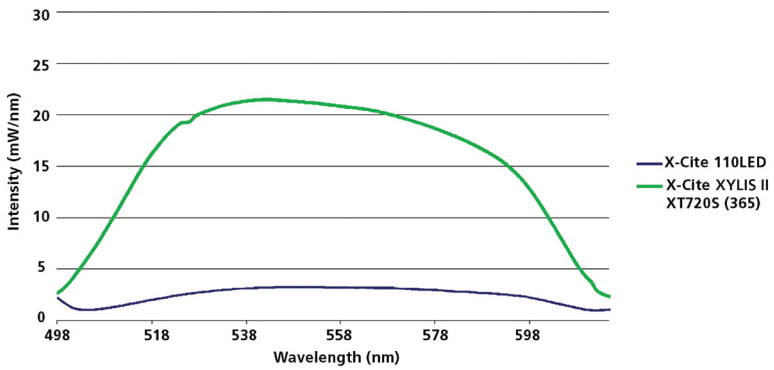
- Trusted X-Cite® brand for superior image illumination.
- Bright fluorescence signals, consistent over the long-term; no need to replace lamps.
- Instant ON/OFF eliminates the need to wait for the system to warm up or cool down before it is ready to use.
- 1% intensity control with convenient hand controller allows each user to set a comfortable intensity level to help reduce eyestrain while working.
- Whisper quiet operation.
- Optional foot pedal is available.

### Comparison of Power

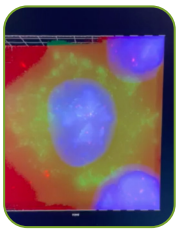



XYLIS II offers two options for DAPI excitation at 365 nm or 385 nm. Users should choose the version best suited to their fluorophore excitation spectrum.

### Green Gap




XYLIS II optical power (green curve) is increased significantly with LaserLED Hybrid Drive technology when compared to a normal green LED (dark blue curve).



*To view a video of cancer cells courtesy of Dr. Yulia Shifrin, Telo Genomics Corp., click the link below.*

https://www.youtube.com/shorts/6Yip763BbjQ

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