Simultaneous Visualization of 16 DNA Loci in Single Cells using the SPECTRA Light Engine

Fundamental processes including DNA replication, transcription, and DNA repair are controlled to some extent by the three-dimensional (3D) organization of the genome. Single-molecule DNA fluorescence *in situ* hybridization (FISH) techniques, used to map spatial relationships of DNA loci, use molecular barcodes to identify DNA sequences. The barcodes may be read either cumulatively, during multiple cycles of hybridization and imaging [1], or in parallel. Parallel detection of 16 DNA loci evenly spaced on human chromosome 2 in a single round of hybridization and imaging (Figure 1) has been demonstrated by Dr. Magda Bienko and colleagues from the Karolinska Institute in Stockholm [2]. The required 16 spectral barcodes were generated by combinatorial labeling using 6 fluorophores excited by a customized SPECTRA Light Engine (Figure 2). Hoechst 33342 was used for cell cycle discrimination based on total nuclear DNA content. Inter-DNA locus distances were determined by multicolor FISH. Distances were validated by demonstration of inverse scaling with chromosome contact frequencies determined by high-throughput chromosome conformation capture (Hi-C).

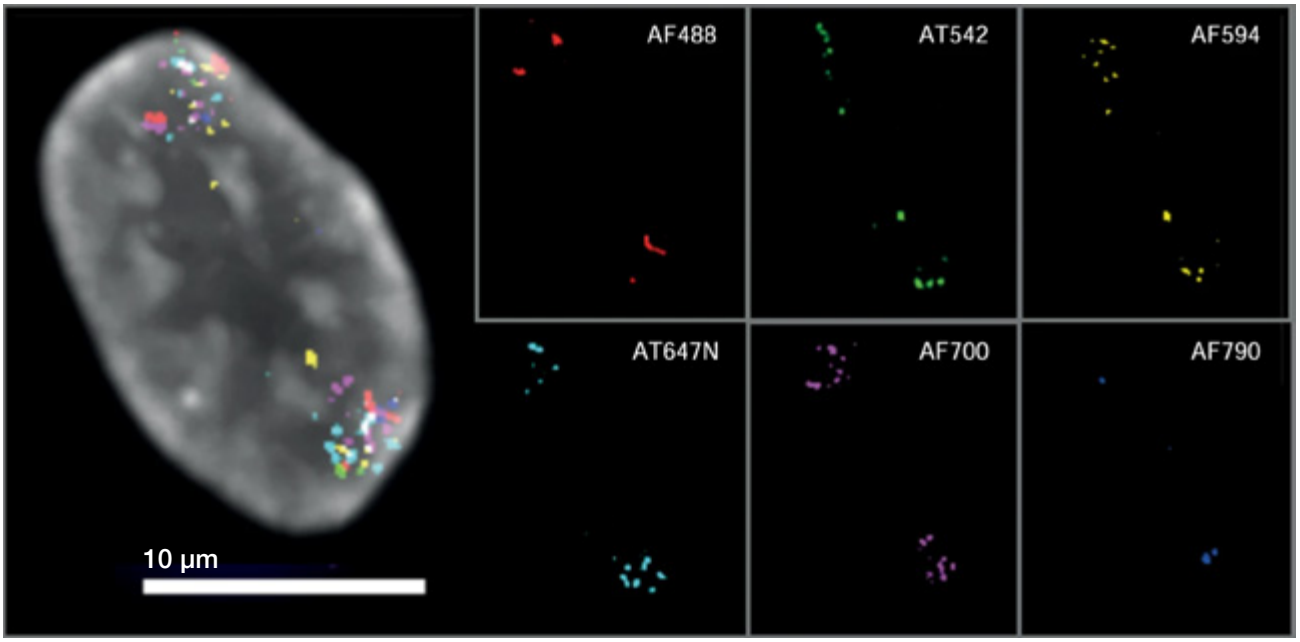

Figure 1. Localization of 16 FISH probes in a single retinal pigment epithelial (RPE) cell nucleus. Individual images for each of the 6 fluorophores used to construct the combinatorially color encoded composite image are shown in the panels on the right. Gray represents DNA stained with Hoechst 33342. Reproduced from [2] under CC BY 4.0.

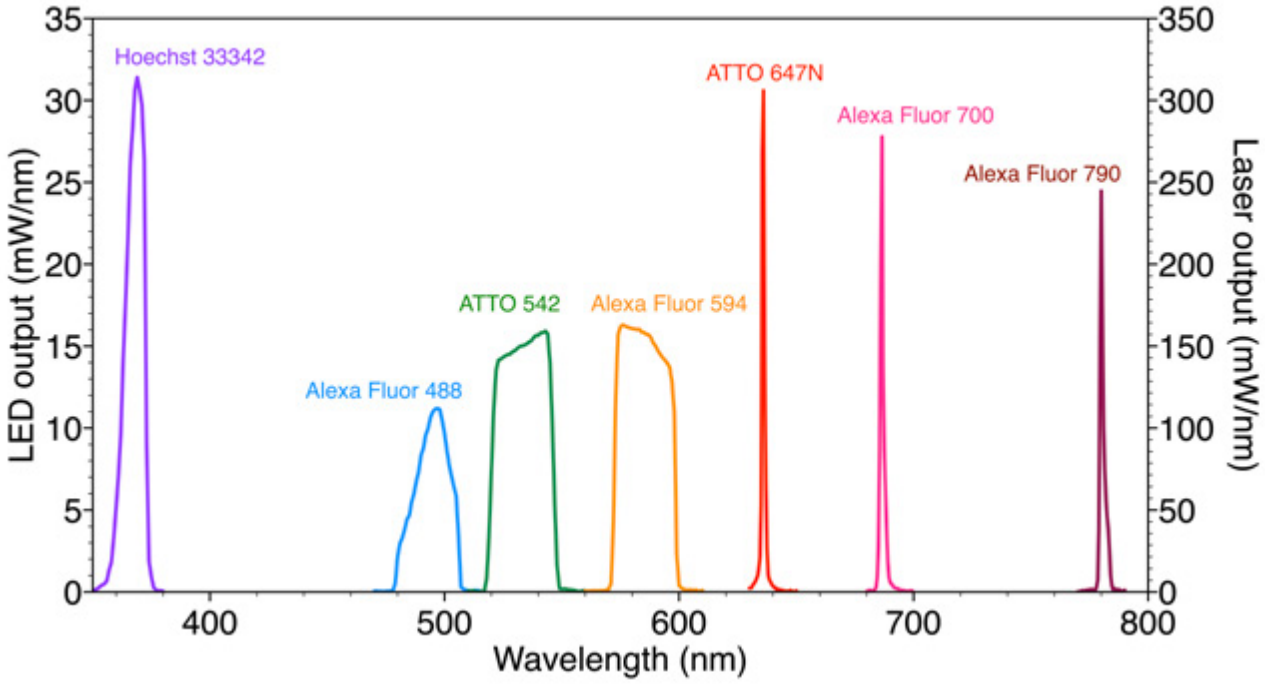

Figure 2. Spectral output of customized SPECTRA light engine for single-molecule FISH localization of 16 DNA loci [2].

References

[1] JH Su, P Zheng, X Zhuang et al. (2020) Cell 182:1641–1659
[2] A Mota, M Schweitzer, M Bienko et al, (2022) Sci Data 9:47