Motic®

MORE THAN MICROSCOPY

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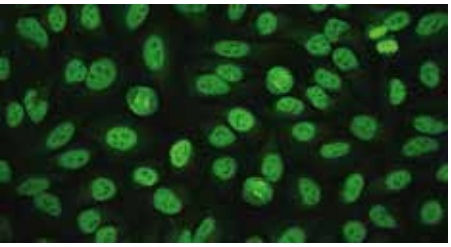
FLUORESCENCE

BASIC NOTES ON FLUORESCENCE



A COLORFUL EXPRESSION

Fluorescence is a molecular phenomenon of spontaneous light emission from a substance after being excited. The emitted light is always of lower energy (longer wavelength) than the excitation energy.

The excitation/emission process is extremely sensible, making fluorescence the most complex contrast method in widefield microscopy. An incorrect setup may cause more than a bad image: there might be no visible signal at all.

A SIMPLIFIED APPROACH TO FLUORESCENCE

- Specific dyes have been developed to selectively stain parts of a specimen. These dyes are called fluorochromes.
- A selected wavelength is applied to the sample (excitation); this wavelength refers to the absorption maximum of the fluorochrome.
- Energy is absorbed by the fluorochrome, shifting it to a higher energy level.
- The excited state of the fluorochrome lasts only a split second; falling back to the original energy level is accompanied by an emission of light.
- As the emission comes along with a partial energy transformation, all emission is less energetic than its corresponding excitation.
- "Stokes shift" describes this effect: The emission is always shifted to a longer wavelength (color).

UV excitation → **BLUE** emission
BLUE excitation → **GREEN** emission
GREEN excitation → **RED** emission

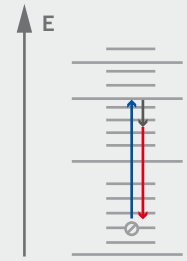
As the energy relation of excitation to emission is 10.000:1, a maximum efficient hardware setup has to be targeted.

The Mercury bulb as fluorescence light source ensures a broad energy spectrum for excitation from 300nm to 600nm, including several intensity peaks.

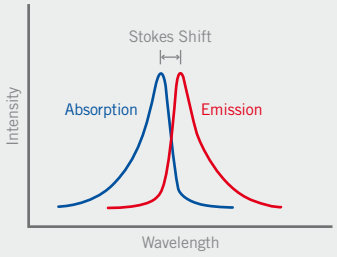
To optimize the performance, specific objectives with maximum Numerical Apertures and maximum transmission (especially in UV) are recommended. A good choice is Motic's selection of PLAN FLUAR PL FL objectives.

Long working distance (LWD) objectives and Phase contrast lenses may be necessary because of sample characteristics, but will not allow the brightest possible signal.

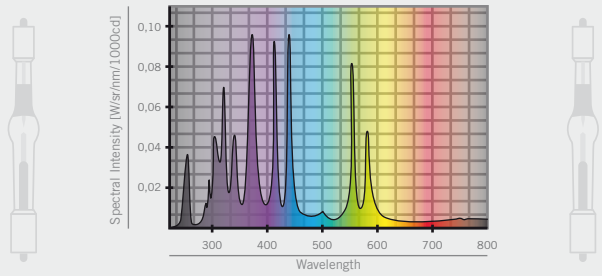
Principle of excitation/emission



Stokes Shift



Mercury Arc Lamp Spectral Distribution



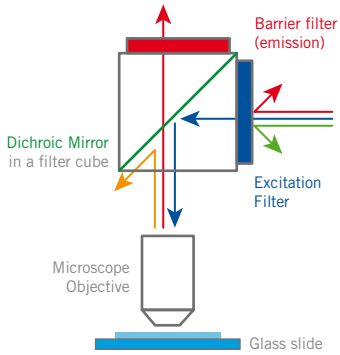
PLAN FLUAR OBJECTIVES

Objectives	N.A.	W.D. (mm)
Plan Fluar PL FL 4X	0.13	20.5
Plan Fluar PL FL 10X	0.30	10.5
Plan Fluar PL FL 20X	0.50	1.9
Plan Fluar PL FL 40X	0.75	0.58
Plan Fluar PL FL 50X	1.0 Oil	0.17
Plan Fluar PL FL 100X	1.3 Oil	0.20

Motic's fluorescence filter cubes can be used on both upright and inverted microscopes.
Following the microscope type, the fluorescence ray path requests a different filter cube orientation.

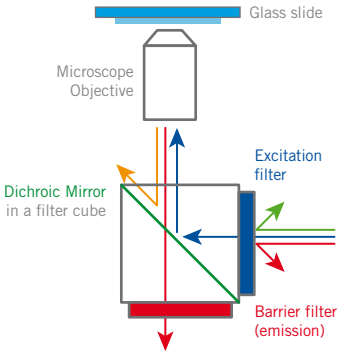
UPRIGHT MICROSCOPE - BA410E

FILTER CUBE ORIENTATION



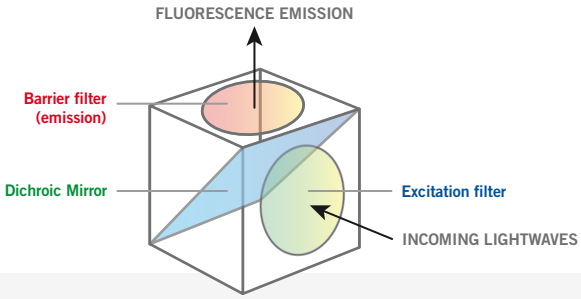
INVERTED MICROSCOPE - AE31E

FILTER CUBE ORIENTATION



A standard Fluorescence filter cube consists of a combination of 3 independent yet inter-coordinated filters:

- **Excitation filter**
- **Dichroic Mirror**
- **Barrier filter (emission)**



The excitation filter has a band pass characteristics, opening a defined sector within the spectrum of the Mercury light source to match the absorption peak of the fluorochrome.

In a given filter specification, the excitation peak of the filter is specified by the first numeric value, while the width of the excitation sector at 50% of the maximum intensity is displayed by the second number (peak width at half height).

A value of 480/30 (nm) for FITC describes a curve of excitation from 465 to 495nm, with a peak around 480nm.The broader the sector, the more energy will excite the sample. A broad sector will result in a strong but unspecific excitation. A narrow sector effectuates a more specific excitation. Filters with narrow excitations are especially desired in case of multiple staining, where excitation maxima of diverse fluorochromes (e.g. GFP mutants) may be close to each other.

The dichroic mirror (from the Greek dikhroos, meaning two-colored) is a long pass filter which starts transmission from the specified wavelength on (e.g.505nm in FITC). For shorter wavelengths the dichroic is impervious and thus acts as a mirror. So the separation of excitation light and emission signal is achieved.

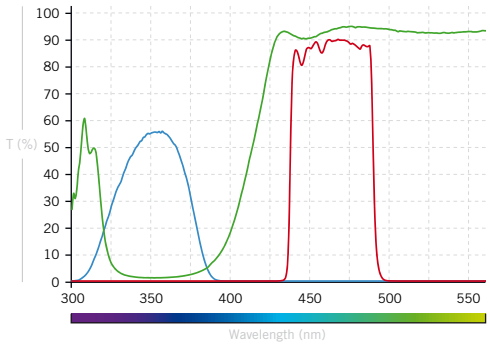
The emission (also called barrier) filter may be designed with long pass or band pass characteristics. The long pass displays all emissions from the stated wavelength on (515nm in FITC), resulting in a mixed color image, while the band pass construction cuts out a pure color. Multiple staining always requests band pass emission filters, as the single signals have to be separated.

To select the correct filter cube, the applied dyes and their excitation/emission maxima have to be carefully taken into consideration. A standard equipment may include DAPI, FITC and TRITC filter cubes, which will cover common dyes in clearly separate sectors of the Mercury bulb spectrum.

MOTIC'S STANDARD FILTER COMBINATIONS

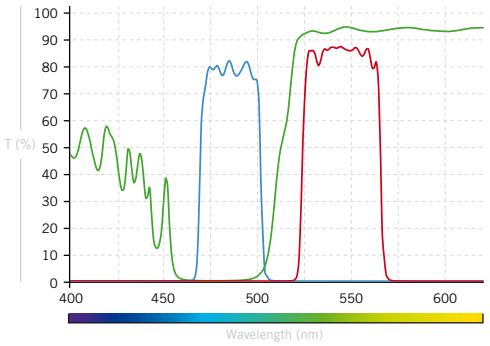
DAPI / Hoechst

Exciter D350/50X Dichroic 400DCLP Barrier D460/50m



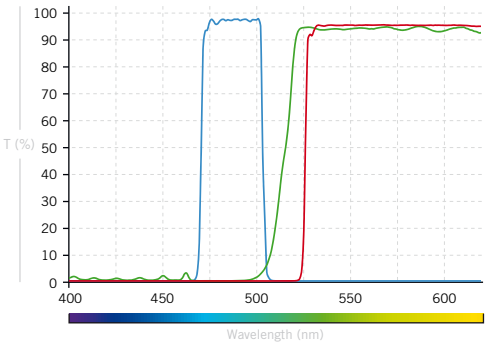
FITC / RSGFP / Fluo3

Exciter D480/30X Dichroic 505DCLP Barrier D535/40m



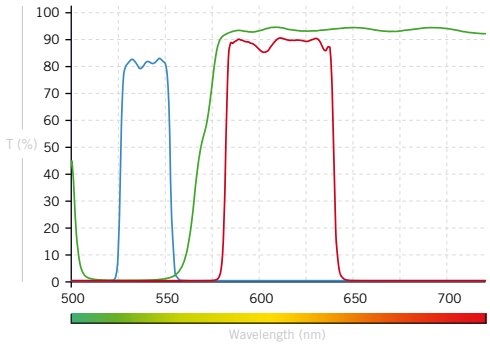
FITC Long pass

Exciter D470/40X Dichroic 505DCLP Barrier E515LPv2



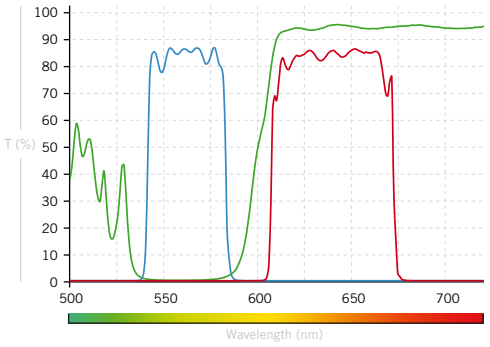
TRITC (Rhodamine) / CY3

Exciter D540/25X Dichroic D565DCLP Barrier D605/55m



Texas Red / Cy3.5

Exciter D560/40X Dichroic 595DCLP Barrier D630/60m

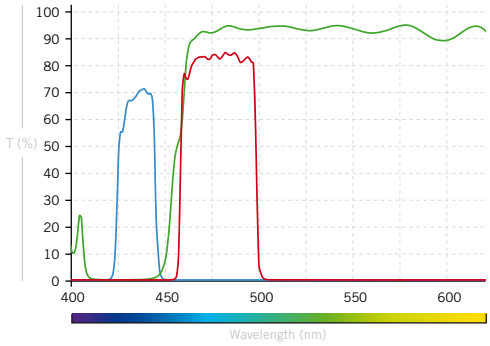


Cy5 / Fluor 633 / Alexa Fluor 647

Exciter HQ620/60X Dichroic Q660LP Barrier HQ700/75m

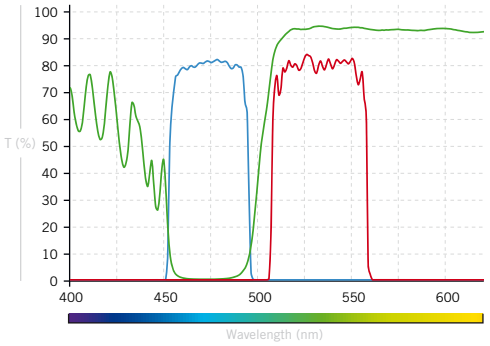
Cyan GFP

Exciter D436/20X Dichroic 455DCLP Barrier D480/40m



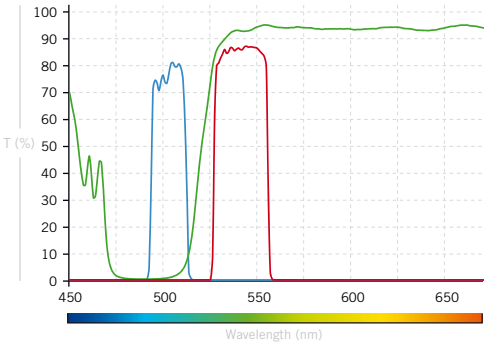
Endow GFP

Exciter HQ470/40X Dichroic Q495LP Barrier HQ525/50m



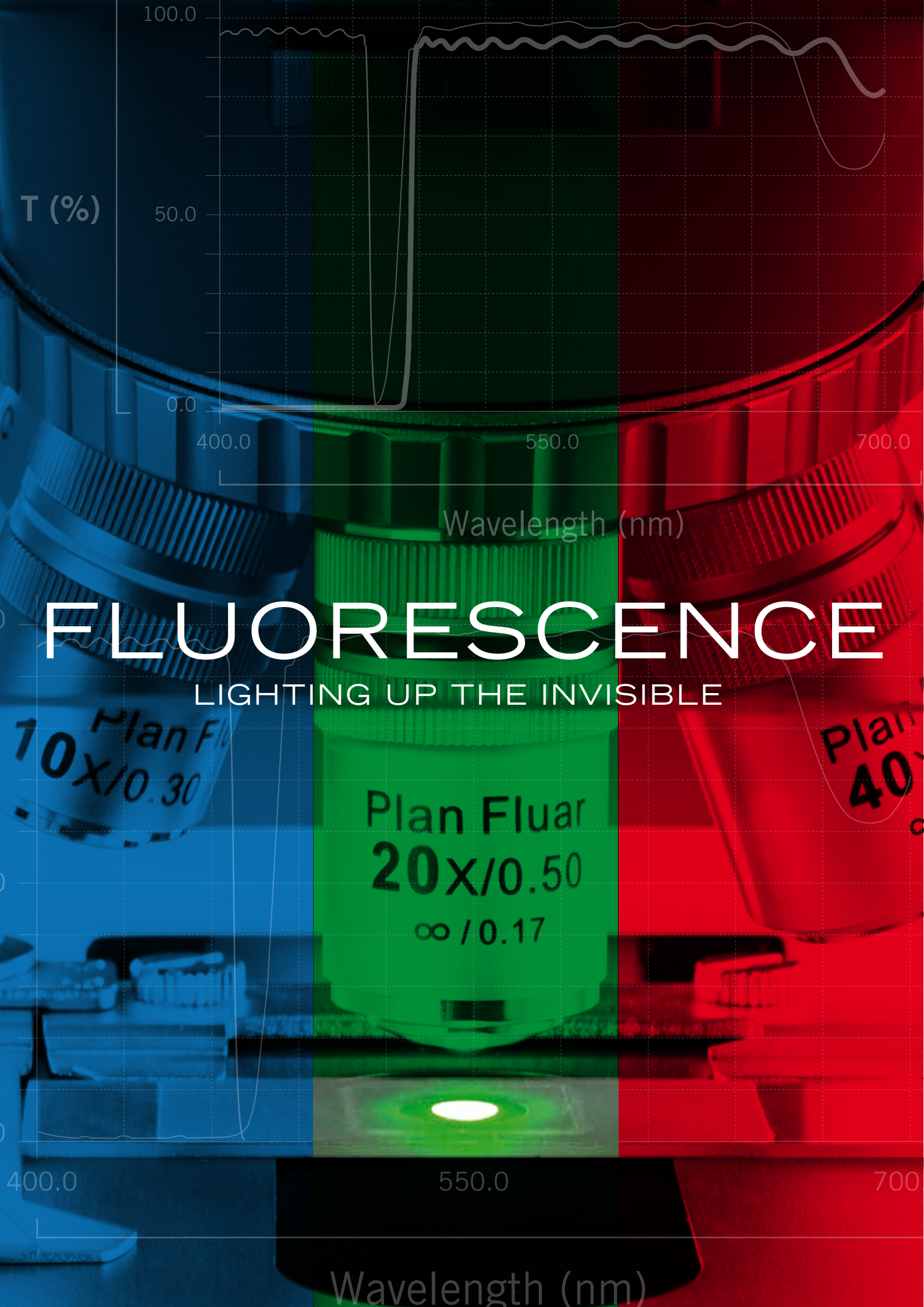
Yellow GFP

Exciter HQ500/20X Dichroic Q515LP Barrier HQ535/30m



MOTIC'S STANDARD FILTER COMBINATIONS ARE:

Filter combinations	Exciter	Dichroic	Barrier Filter
DAPI/Hoechst	D350/50X	400DCLP	D460/50m
FITC/RSGFP/Fluo3	D480/30X	505DCLP	D535/40m
FITC Long Pass	D470/40X	505DCLP	E515LPv2
TRITC (Rhodamine) / CY3	D540/25X	D565DCLP	D605/55m
Texas Red/Cy3.5	D560/40X	595DCLP	D630/60m
Cy5/Fluor633/Alexa Fluor 647	HQ620/60X	Q660LP	HQ700/75m
Cyan GFP	D436/20X	455DCLP	D480/40m
Endow GFP	HQ470/40X	Q495LP	HQ525/50m
Yellow GFP	HQ500/20X	Q515LP	HQ535/30m



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LIGHTING UP THE INVISIBLE

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