The excitation and emission spectra of the Alexa Fluor series cover the visible spectrum and extend into the infrared . ^[2] The individual members of the family are numbered according roughly to their excitation maxima (in nm).

Alexa Fluor dyes are synthesized through sulfonation of coumarin, rhodamine, xanthene(such as fluorescein), and cyanine dyes. Sulfonation makes Alexa Fluor dyes negatively charged and hydrophilic. Alexa Fluor dyes are generally more stable, brighter, and less pH-sensitive than common dyes (e.g. fluorescein, rhodamine) of comparable excitation and emission, [3] and to some extent the newer cyanine series.^[4] However, they are also more expensive. They are patented by Invitrogen (which acquired the company that developed the Alexa dyes, Molecular Probes) and thus are priced higher than the common dyes that are available from multiple manufacturers.

Similar alternatives include the <u>DyLight Fluors</u> from Pierce (Thermo Fisher Scientific), and the Atto series from Atto-Tec and sold by Sigma-Aldrich

Alexa	blue	346	442	410	10 000
Fluor	blue	340	442	410	19,000
350					
– 405	violet	401	421	1028	34,000
- 430	green	434	541	702	16,000
- 488	green	495	519	643	71,000
— 500	green	502	525	700	71,000
- 514	green	517	542	714	80,000
— 532	green	532	554	721	81,000
– 546	yellow- green	556	573	1079	104,000
— 555	green	555	565	~1250	150,000
- 568	orange	578	603	792	91,300
— 594	orange- red	590	617	820	90,000
- 610	red	612	628	1172	138,000
- 633	not vis	632	647	~1200	100,000
— 647	not vis	650	665	~1300	239,000
- 660	not vis	663	690	~1100	132,000
- 680	not vis	679	702	~1150	184,000
— 700	not vis	702	723	~1400	192,000
— 750	not vis	749	775	~1300	240,000
↑ = approximate color of the emission spectrum					

 $\varepsilon = \underbrace{\text{extinction coefficient}}$

Comparison with other dyes

The Alexa series dyes are less pH-sensitive and more photostable than the original dyes (fluorescein, rhodamine, etc.) from which they were synthesized.

However, brightness comparisons are not presently available. Brightness is commonly measured as a product of extinction coefficient (absorption efficiency) and quantum yield (emission efficiency) [5] While extinction coefficients are known (see the table above), the quantum yields of the Alexas have not been published by Molecular Probes.

In one case, a third party has compared one Alexa with another commonly used dye. This was a comparison of Cy5 and its Alexa with similar wavelength, Alexa 647, with the dyes conjugated to DNA. [1] This study found that Cy5 is brighter, but less photostable than Alexa 647.

Therefore presently it is difficult to quantitatively choose the best dye for a particular application and in many cases empirical testing is in order.

- 1. Alexa Fluor Dyes Spanning the Visible and Infrared Spectrum (2007-06-06). Retrieved on 2007-08-13.
- 2. The Alexa Fluor Dye Series. Molecular Probes, Inc. (2006-04-06). Retrieved on 2007-08-13.
- 3. Panchuk-Voloshina N, Haugland RP, Bishop-Stewart J, et al (1999). "Alexa dyes, a series of new fluorescent dyes that yield exceptionally bright, photostable conjugates". J. Histochem. Cytochem. 47 (9): 1179-88. PMID 10449539.
- 4. Berlier JE, Rothe A, Buller G, et al (2003). "Quantitative comparison of long-wavelength Alexa Fluor dyes to Cy dyes: fluorescence of the dyes and their bioconjugates". J. Histochem. Cytochem. 51 (12): 1699-712. PMID 14623938.
- 5. Souslova EA, Belousov VV, Lock JG, Stromblad S, Kasparov S, Bolshakov AP, Pinelis VG, Labas YA, Lukyanov S, Mayr LM, Chudakov DM (2007). "Single fluorescent protein-based Ca2+ sensors with increased dynamic range". BMC Biotechnol. 7 (7): 37. PMID 17603870.