2) Setup

- bright light source, high emission in the short wave length regime

  e.g. mercury burner (HBO 50, HBO 100)
  xenon burner (XBO 75)

usually epifluorescence:
excitation and emission light uses the light path through objective

- excitation filter, to selectively select a fluorophore
- dichroic mirror:
  - allows ~~emission~~ excitation light to be directed from the light source to the objective
  - allows emission light to pass through the mirror and to be directed into the direction of the eye piece
- emission filter:
  fluorescence is a weak effect
  thus the excitation light has to be blocked

- filters:

interference filters (absorption filters usually out dated)

Broad band: high photon yield, unspecific background

small band: low photon yield, and background

⇒ optimize for signal-to-noise ratio

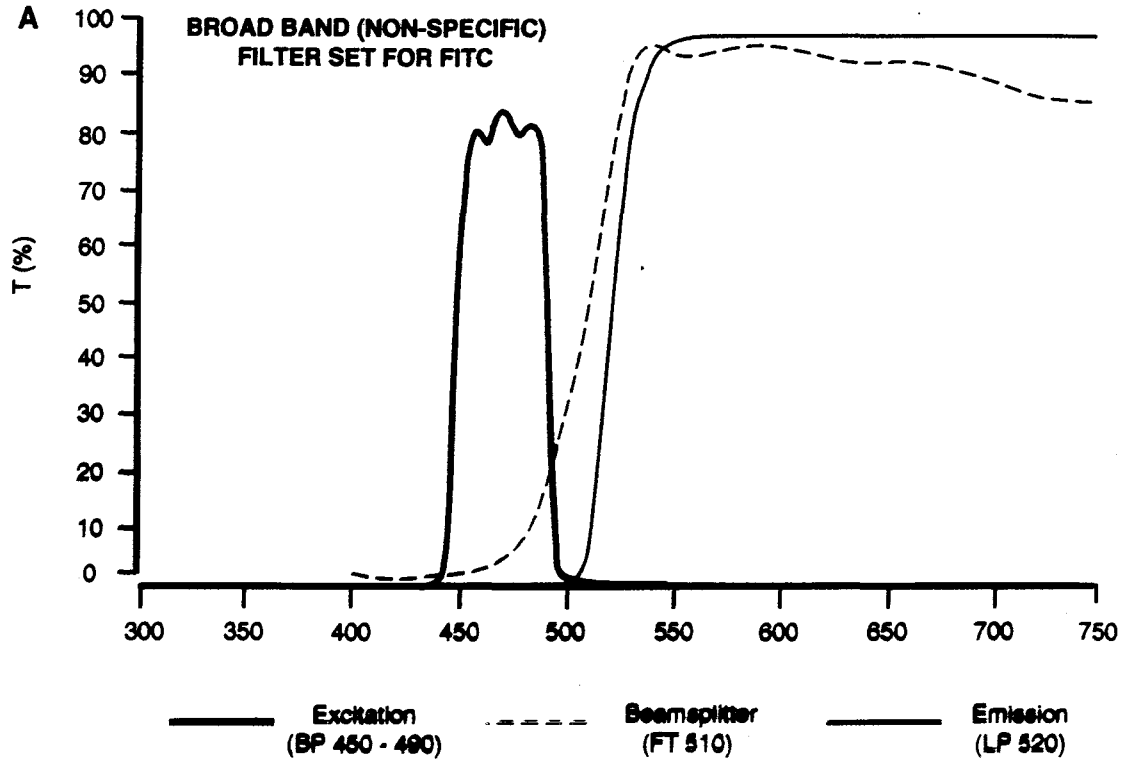
multi-colour fluorescence ⇒ filter wheels

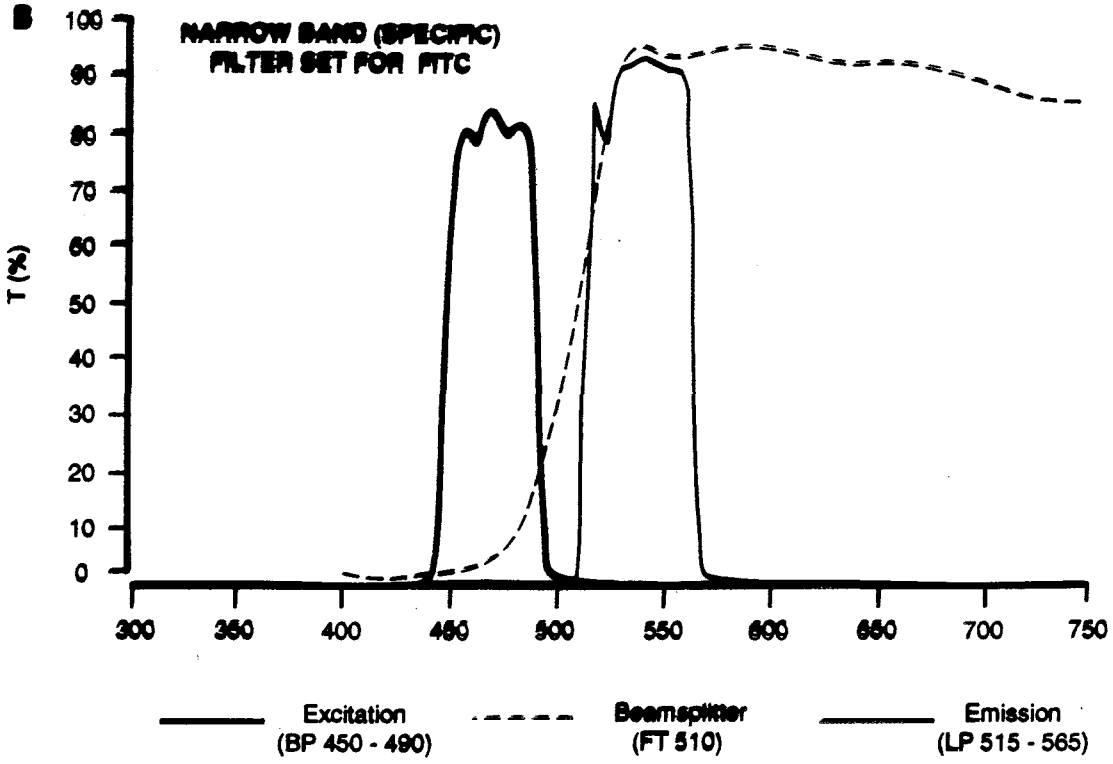
- objectives:

- relative image brightness $B = \frac{N_{A\,objective}^4}{f_{Microscope}^2}$

- chromatic colour correction not important since monochromatic technique

- objectives with high aperature & and minimal necessary magnification (often oil immersion)


A
BROAD BAND (NON-SPECIFIC)
FILTER SET FOR FITC
T (%)
100
90
80
70
60
50
40
30
20
10
0
300
350
400
450
500
550
600
650
700
750
Excitation
(BP 450 - 490)
Beamsplitter
(FT 510)
Emission
(LP 520)


B
NARROW BAND (SPECIFIC)
FILTER SET FOR FITC
T (%)
100
90
80
70
60
50
40
30
20
10
0
300
350
400
450
500
550
600
650
700
750
Excitation
(BP 450 - 490)
Beamsplitter
(FT 510)
Emission
(LP 515 - 565)

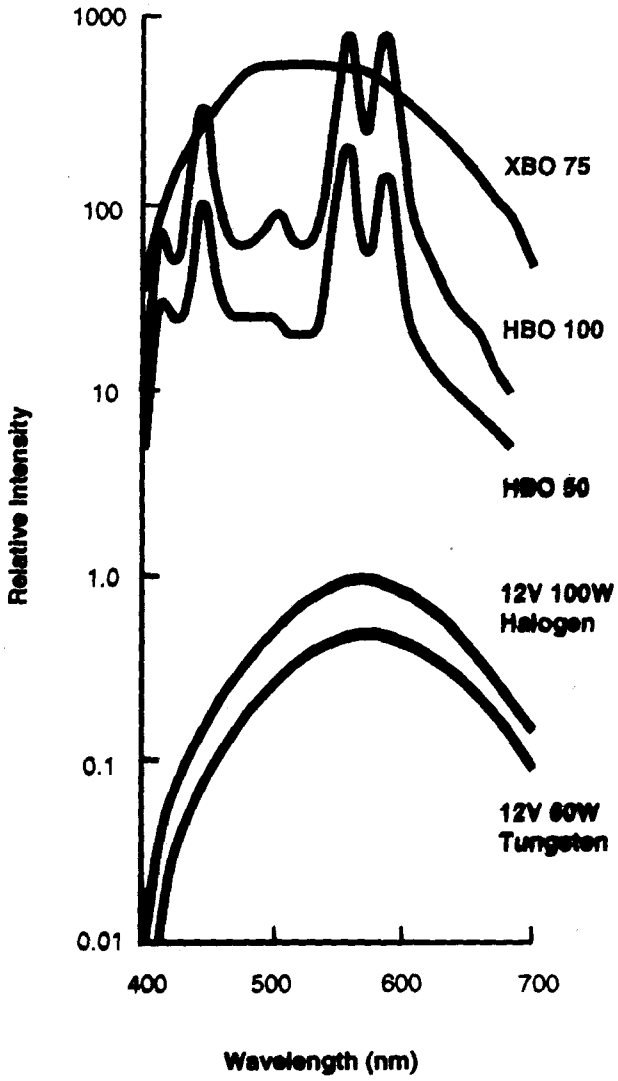


FIGURE 94.31
Relative spectral intensity of various light sources with heat reflection filter in light path.

stances, proper focus and alignment are particularly critical (for further details, see the discussion on fluorescence microscopy above). To ionize the internal gas, discharge lamps are ignited with high-voltage pulses provided by their power supply. They reach their full intensity after several minutes and then operate at high internal pressure (10–15 atmospheres) and must be used in a safe housing that provides adequate cooling. Follow the manufacturer's instructions on safety, installation, and alignment closely! Figure 94.31 shows the relative spectral output for a range of different light sources.

Where the intensity or high luminous density of a 100 W HBO is needed along with uniform filling of the large condenser aperture, as in high-resolution DIC, a so-called Ellis scrambler (after Gordon Ellis, University of Pennsylvania) makes an ideal interface between source and microscope: A quartz fiber approximately 1 m long and 1 mm in diameter receives the nonuniform arc at its entry through special collector optics. Giving the fiber a loop, the exit of the fiber becomes a round uniform source of high intensity that through additional optics can be enlarged to fill

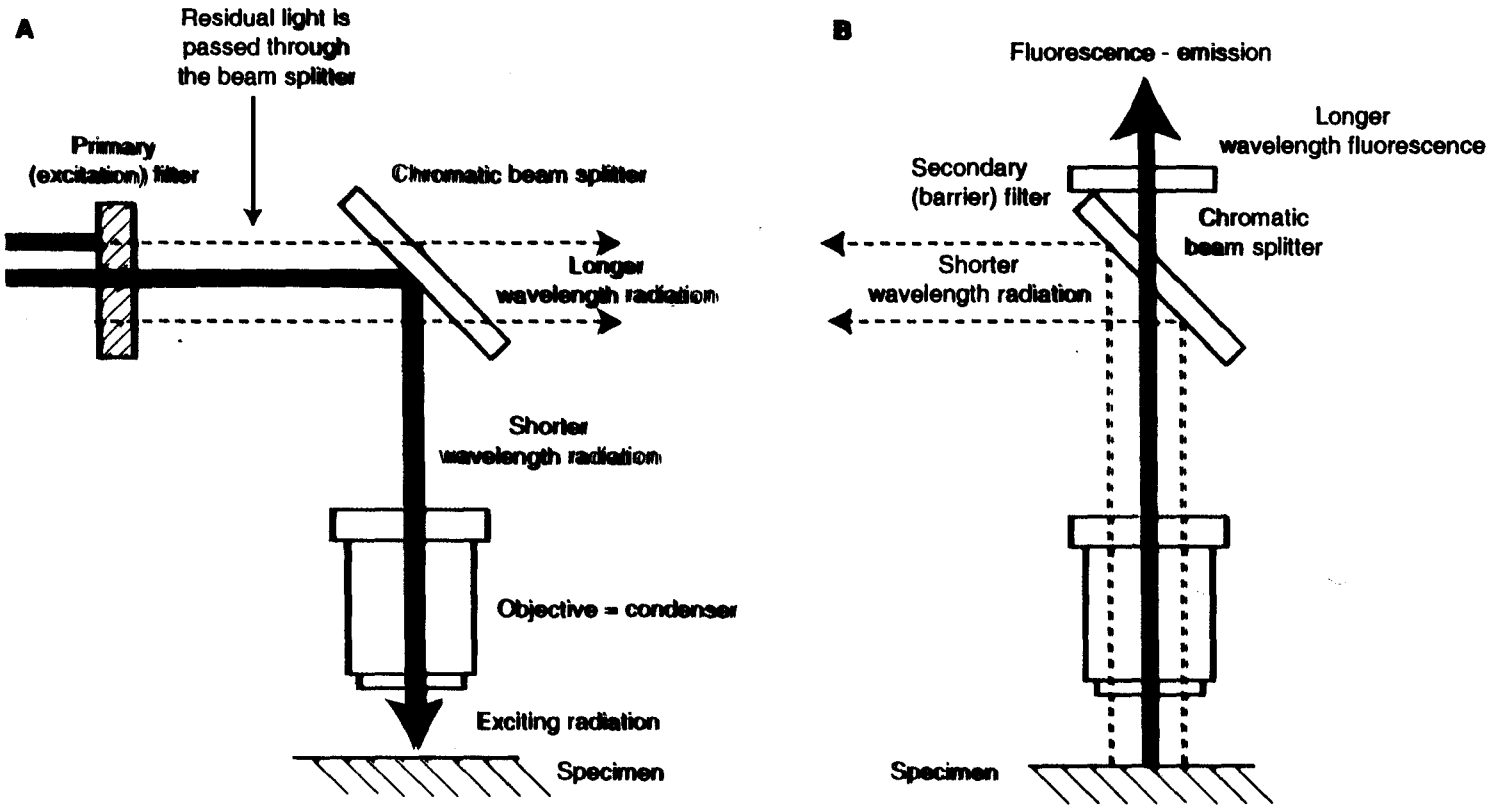


FIGURE 94.25
Filter components of the epifluorescence microscope. The blue excitation light and the green light emitted from the specimen are typical for the fluorochrome fluorescein.