Multiedge dichroic analysis

If we could use a five-edge dichroic, we would significantly reduce the complexity of the instrument for wide field fluorescence. On the other hand, this is relatively expensive (Laser2000 suggest ~10k for Semrock, Chroma waiting for whether we're satisfied with spec before providing a quote) and locks us in to some extent to the current set of fluorophores. Perhaps most significantly, the increased complexity of the fabrication process will result in a performance drop in terms of signal in any one band.

How bad is this performance drop?

First, import files defining the spectral characteristics of the system.

In [1]:

```
import os
import numpy as np
import matplotlib.pyplot as plt
# from bokeh.io import push_notebook, show, output_notebook
# from bokeh.plotting import figure
import seaborn as sns

from dye import Dye
from laser import Laser
from filterCube import FilterCube
from camera import Camera
from objective import Objective
import utils
from crosstalk_analysis import signalFromDyeXInChannelY
from crosstalk_analysis import displayCrosstalkPlot

dyesPath = 'Dye spectra/'
filtersPath = 'Filter spectra/'
opticsPath =  'Optics spectra/'
cameraPath = 'Camera spectra/'

%matplotlib inline
# output_notebook()
```

In [2]:

```
def show_dye_emission_enclosed_by_filters(dye, filtercube, objective, camera, title='du
mmy title'):
    """Given dye and filtercube, give visual indication of overlap"""

    dem = utils.interpolateSpectrum(dye.emissionSpectrum, 0.5)
    fem = utils.interpolateSpectrum(filtercube.emissionFilter.getSpectrum(), 0.5)
    fdiem = utils.interpolateSpectrum(filtercube.dichroicFilter.getSpectrum(), 0.5)
    spectra = [dem, fem, fdiem]

    lowerLimit = max( [min(spectrum[:,0]) for spectrum in spectra] )
    upperLimit = min( [max(spectrum[:,0]) for spectrum in spectra] )

    trimmedSpectra = [spectrum[(spectrum[:,0] >= lowerLimit) & (spectrum[:,0] <= upperL
imit)] for spectrum in spectra]

    ovrlp = np.ones((trimmedSpectra[0][:,1].shape))

    for spectrum in trimmedSpectra:
        ovrlp = np.multiply(ovrlp, spectrum[:,1])

    hfig = plt.figure()
    plt.title(filtercube.channel + ' ' + title)
    utils.displaySpectra(trimmedSpectra)
    plt.fill_between(trimmedSpectra[0][:,0], ovrlp)

    return np.sum(ovrlp)*0.5
```

Sources for dye data:

Atto general properties: https://www.atto-tec.com/fileadmin/user_upload/Katalog_Flyer_Support/Dye_Properties_01.pdf (https://www.atto-tec.com/fileadmin/user_upload/Katalog_Flyer_Support/Dye_Properties_01.pdf)

Atto spectra: https://www.atto-tec.com/attotecshop/product_info.php?language=en&info=p117_ATTO-700.html (https://www.atto-tec.com/attotecshop/product_info.php?language=en&info=p117_ATTO-700.html)

Alexa 405 absorption coefficient: http://www.atdbio.com/content/34/Alexa-dyes (http://www.atdbio.com/content/34/Alexa-dyes)

Alexa 405 QY: http://confocal-microscopy-list.588098.n2.nabble.com/alexa-405-QY-td6913848.html (http://confocal-microscopy-list.588098.n2.nabble.com/alexa-405-QY-td6913848.html)

Alexa 405 spectra: https://www.chroma.com/spectra-viewer?fluorochromes=10533 (https://www.chroma.com/spectra-viewer?fluorochromes=10533)

Sources for filter spectra:

Semrock filters: http://www.laser2000.co.uk (http://www.laser2000.co.uk)

Chroma 700 dichroics: emailed from Chroma

Sources for camera spectra:

Andor Zyla 5.5: https://searchlight.semrock.com/ (https://searchlight.semrock.com/)

Sources for optics (objective) spectra:

UPLANSAPO20x: https://www.olympus-lifescience.com/en/objectives/uplsapo/#!cms[tab]=%2Fobjectives%2Fuplsapo%2F20x (https://www.olympus-lifescience.com/en/objectives/uplsapo/#!cms[tab]=%2Fobjectives%2Fuplsapo%2F20x), data extracted from curve using WebPlotDigitiser: http://arohatgi.info/WebPlotDigitizer/app/ (http://arohatgi.info/WebPlotDigitizer/app/)

Next, define (manually and in a boilerplate-y manner) the components of the system we are investigating.

In [3]:

```
dye405 = Dye(name = 'Alexa405', epsilon = 35000, qy = 0.54,
             absSpectrum = os.path.join(dyesPath, 'Alexa405abs.txt'),
             emSpectrum = os.path.join(dyesPath, 'Alexa405em.txt'))

dye532 = Dye(name = 'Atto532', epsilon = 115000, qy = 0.9,
             absSpectrum = os.path.join(dyesPath, 'ATTO532_PBS.abs.txt'),
             emSpectrum = os.path.join(dyesPath, 'ATTO532_PBS.ems.txt'))

dye594 = Dye(name = 'Atto594', epsilon = 120000, qy = 0.85,
             absSpectrum = os.path.join(dyesPath, 'ATTO594_PBS.abs.txt'),
             emSpectrum = os.path.join(dyesPath, 'ATTO594_PBS.ems.txt'))

dye633 = Dye(name = "Atto655", epsilon = 125000, qy = 0.3,
          absSpectrum = os.path.join(dyesPath, 'ATTO655_PBS.abs.txt'),
          emSpectrum = os.path.join(dyesPath, 'ATTO655_PBS.ems.txt') )

dye700 = Dye(name = "Atto700", epsilon = 120000, qy = 0.25,
          absSpectrum = os.path.join(dyesPath, 'ATTO700_PBS.abs.txt'),
```

```
        emSpectrum = os.path.join(dyesPath, 'ATTO700_PBS.ems.txt') )

l405 = Laser(channel = 'L405Nm', centreWavelengthNm = 405, fwhmNm = 0.01,
             laserOutputPowerMw = 3)

l532 = Laser(channel = 'L532Nm', centreWavelengthNm = 532, fwhmNm = 0.01,
             laserOutputPowerMw = 18)

l594 = Laser(channel = 'L594Nm', centreWavelengthNm = 594, fwhmNm = 0.01,
             laserOutputPowerMw = 25)

l633 = Laser(channel = 'L633Nm', centreWavelengthNm = 640, fwhmNm = 0.01,
             laserOutputPowerMw = 30)

l700 = Laser(channel = 'L700Nm', centreWavelengthNm = 701, fwhmNm = 0.01,
             laserOutputPowerMw = 30)

fc405 = FilterCube(channel = 'L405Nm',
                   excitationFilter = ( 'FF01-390_40', os.path.join(filtersPath, 'FF01-
390_40_Spectrum.txt') ),
                   dichroicFilter = ( 'Di02-R405', os.path.join(filtersPath, 'Di02-R405
_Spectrum.txt') ),
                   emissionFilter = ( 'FF01-452_45', os.path.join(filtersPath, 'FF01-45
2_45_Spectrum.txt') ) )

fc532 = FilterCube(channel = 'L532Nm',
                   excitationFilter = ( 'FF01-532_3', os.path.join(filtersPath, 'FF01-5
32_3_spectrum.txt') ),
                   dichroicFilter = ( 'Di02-R532', os.path.join(filtersPath, 'Di02-R532
_Spectrum.txt') ),
                   emissionFilter = ( 'FF01-562_40', os.path.join(filtersPath, 'FF01-56
2_40_spectrum.txt') ) )

fc594 = FilterCube(channel = 'L594Nm',
                   excitationFilter = ( 'FF01-591_6', os.path.join(filtersPath, 'FF01-5
91_6_Spectrum.txt') ),
                   dichroicFilter = ( 'Di02-R594', os.path.join(filtersPath, 'Di02-R594
_Spectrum.txt') ),
                   emissionFilter = ( 'FF01-647_57', os.path.join(filtersPath, 'FF01-64
7_57_Spectrum.txt') ) )

fc633 = FilterCube(channel = 'L633Nm',
                   excitationFilter = ( 'FF01-640_14', os.path.join(filtersPath, 'FF01-
640_14_spectrum.txt') ),
                   dichroicFilter = ( 'Di02-R635', os.path.join(filtersPath, 'Di02-R635
_Spectrum.txt') ),
                   emissionFilter = ( 'FF01-679_41', os.path.join(filtersPath, 'FF01-67
9_41_Spectrum.txt') ) )

fc700old = FilterCube(channel = 'L700Nm',
                   excitationFilter = ( 'FF01-692_40', os.path.join(filtersPath, 'FF01-
692_40_Spectrum.txt') ),
                   dichroicFilter = ( '725dcxxr', os.path.join(filtersPath, 'Chroma 725
dcxxr.txt') ),
                   emissionFilter = ( 'FF01-795_150', os.path.join(filtersPath, 'FF01-7
95_150_Spectrum.txt') ) )

fc700new = FilterCube(channel = 'L700Nm',
                   excitationFilter = ( 'FF01-692_40', os.path.join(filtersPath, 'FF01-
692_40_Spectrum.txt') ),
                   dichroicFilter = ( '725lpxr', os.path.join(filtersPath, 'Chroma 725l
```

```
pxr.txt') ),
                   emissionFilter = ( 'FF01-747_33', os.path.join(filtersPath, 'FF01-74
7_33_Spectrum.txt') ) )

fc405multi = FilterCube(channel = 'L405Nm',
                   excitationFilter = ( 'FF01-390_40', os.path.join(filtersPath, 'FF01-
390_40_Spectrum.txt') ),
                   dichroicFilter = ( 'Chroma multiedge', os.path.join(filtersPath, 'Ch
roma ZT405-532-594-640-701rpc.txt') ),
                   emissionFilter = ( 'FF01-452_45', os.path.join(filtersPath, 'FF01-45
2_45_Spectrum.txt') ) )

fc532multi = FilterCube(channel = 'L532Nm',
                   excitationFilter = ( 'FF01-532_3', os.path.join(filtersPath, 'FF01-5
32_3_spectrum.txt') ),
                   dichroicFilter = ( 'Chroma multiedge', os.path.join(filtersPath, 'Ch
roma ZT405-532-594-640-701rpc.txt') ),
                   emissionFilter = ( 'FF01-562_40', os.path.join(filtersPath, 'FF01-56
2_40_spectrum.txt') ) )

fc594multi = FilterCube(channel = 'L594Nm',
                   excitationFilter = ( 'FF01-591_6', os.path.join(filtersPath, 'FF01-5
91_6_Spectrum.txt') ),
                   dichroicFilter = ( 'Chroma multiedge', os.path.join(filtersPath, 'Ch
roma ZT405-532-594-640-701rpc.txt') ),
                   emissionFilter = ( 'FF01-647_57', os.path.join(filtersPath, 'FF01-64
7_57_Spectrum.txt') ) )

fc633multi = FilterCube(channel = 'L633Nm',
                   excitationFilter = ( 'FF01-640_14', os.path.join(filtersPath, 'FF01-
640_14_spectrum.txt') ),
                   dichroicFilter = ( 'Chroma multiedge', os.path.join(filtersPath, 'Ch
roma ZT405-532-594-640-701rpc.txt') ),
                   emissionFilter = ( 'FF01-679_41', os.path.join(filtersPath, 'FF01-67
9_41_Spectrum.txt') ) )

fc700multi = FilterCube(channel = 'L700Nm',
                   excitationFilter = ( 'FF01-692_40', os.path.join(filtersPath, 'FF01-
692_40_Spectrum.txt') ),
                   dichroicFilter = ( 'Chroma multiedge', os.path.join(filtersPath, 'Ch
roma ZT405-532-594-640-701rpc.txt') ),
                   emissionFilter = ( 'FF01-747_33', os.path.join(filtersPath, 'FF01-74
7_33_Spectrum.txt') ) )


camera = Camera(name = 'Andor Zyla 5.5',
                qeCurve = os.path.join(cameraPath, 'Camera sCMOS (Andor- Zyla
5.5).txt'))

objective = Objective(name = 'Olympus UPLANSAPO20x 0.75NA',
                      transmissionCurve = os.path.join(opticsPath, 'Olympus UPLANSAPO20
x.txt'))
```

In [5]:

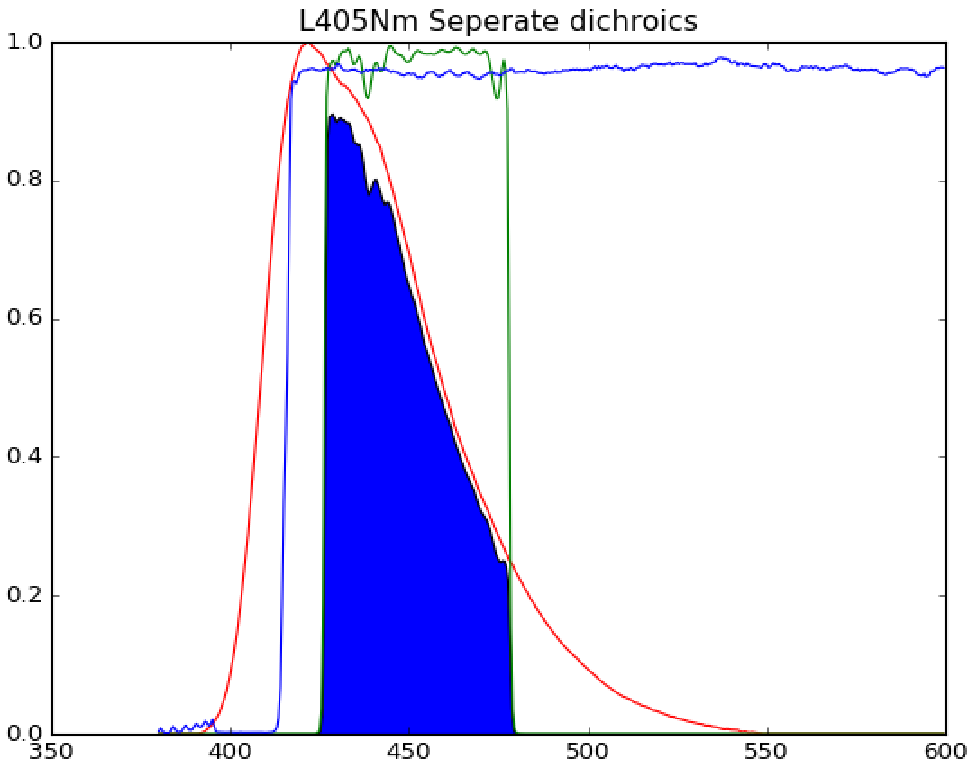
```
laser_list = [l405, l532, l594, l633, l700]
fc_list = [fc405, fc532, fc594, fc633, fc700new]
multi_fc_list = [fc405multi, fc532multi, fc594multi, fc633multi, fc700multi]
dye_list = [dye405, dye532, dye594, dye633, dye700]
```

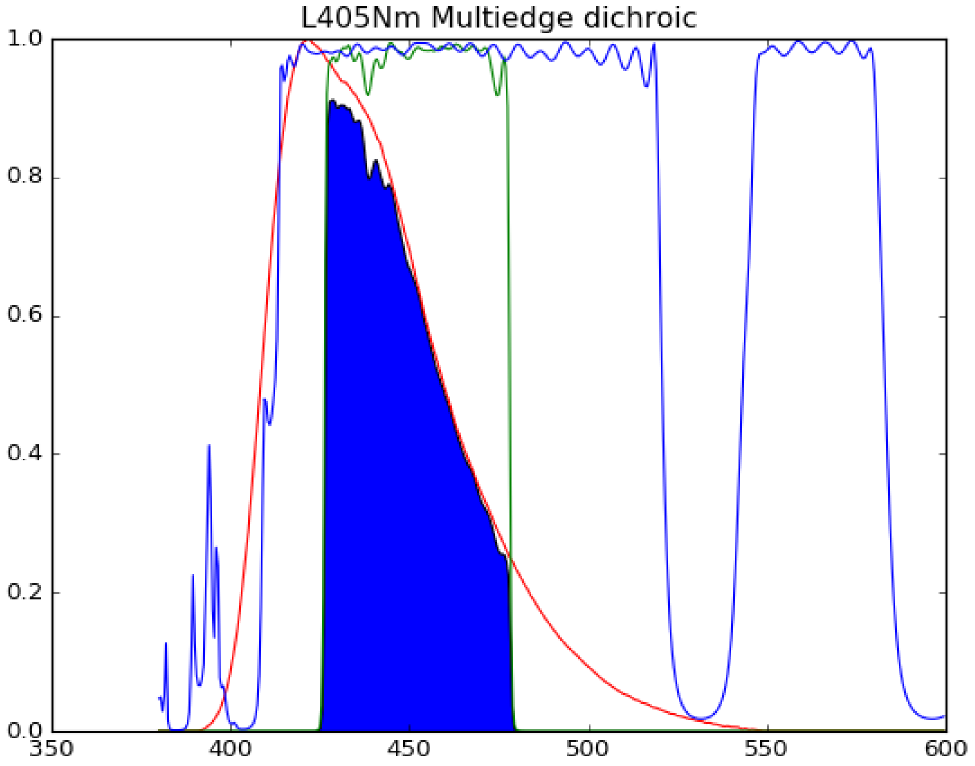
Signal comparison

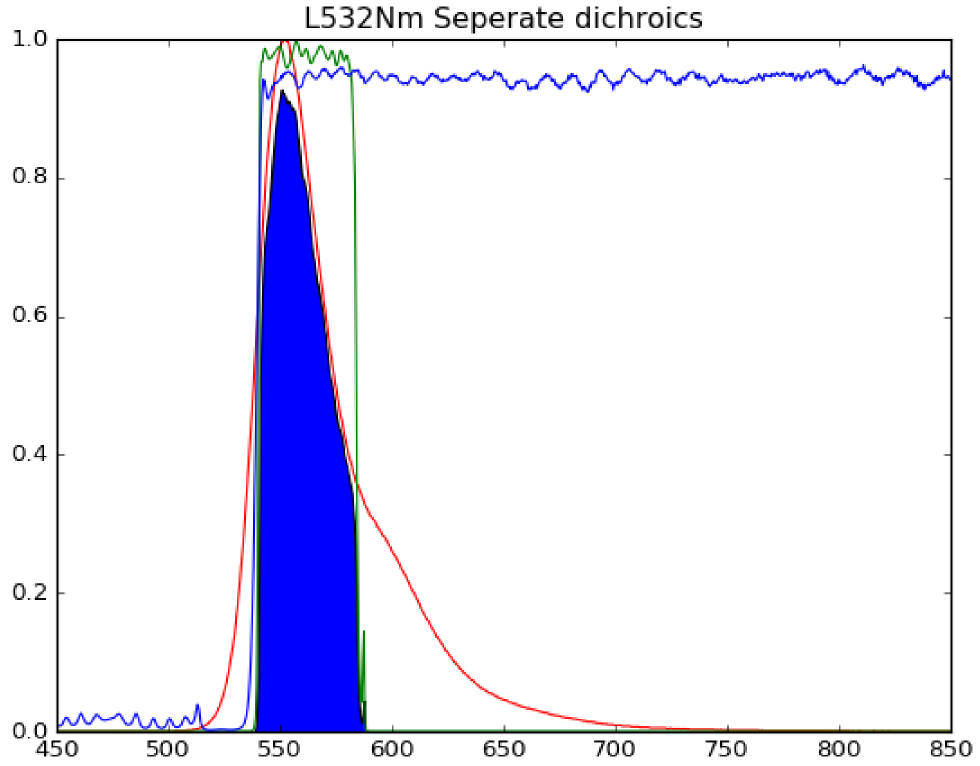
Then consider the relative (correct) signal from each of the dyes in their respective detection channels in the new system compared to the current system. Note that dye concentration is not considered so raw signal cannot be compared between channels, but that is is nevertheless valid to compare the two different setups in each channel individually.

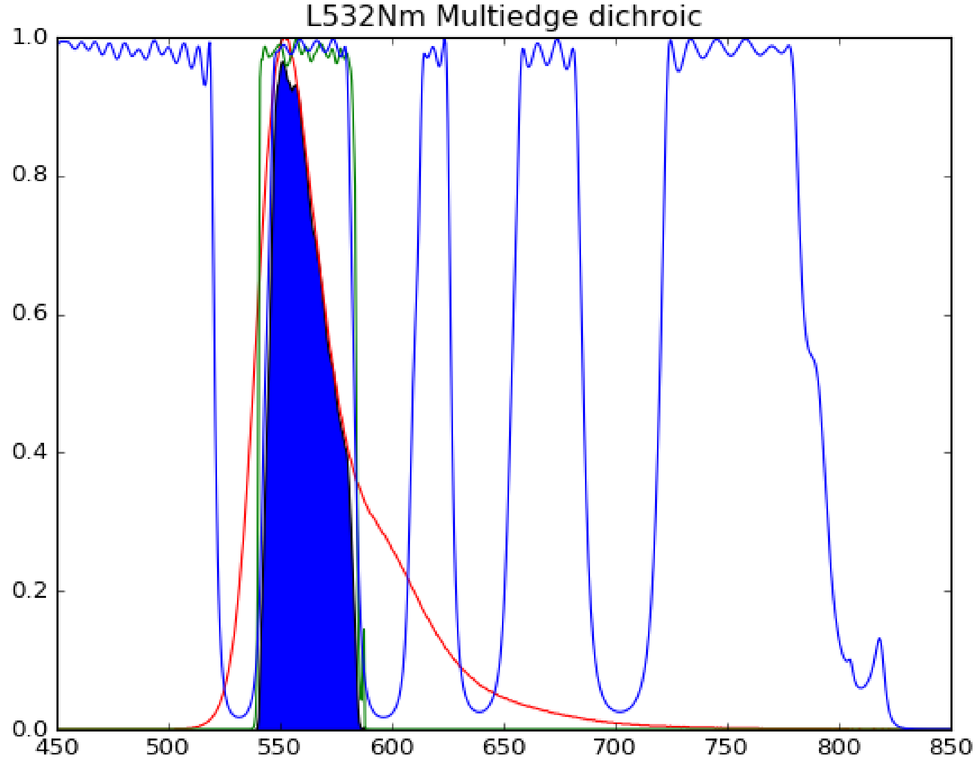
In [6]:

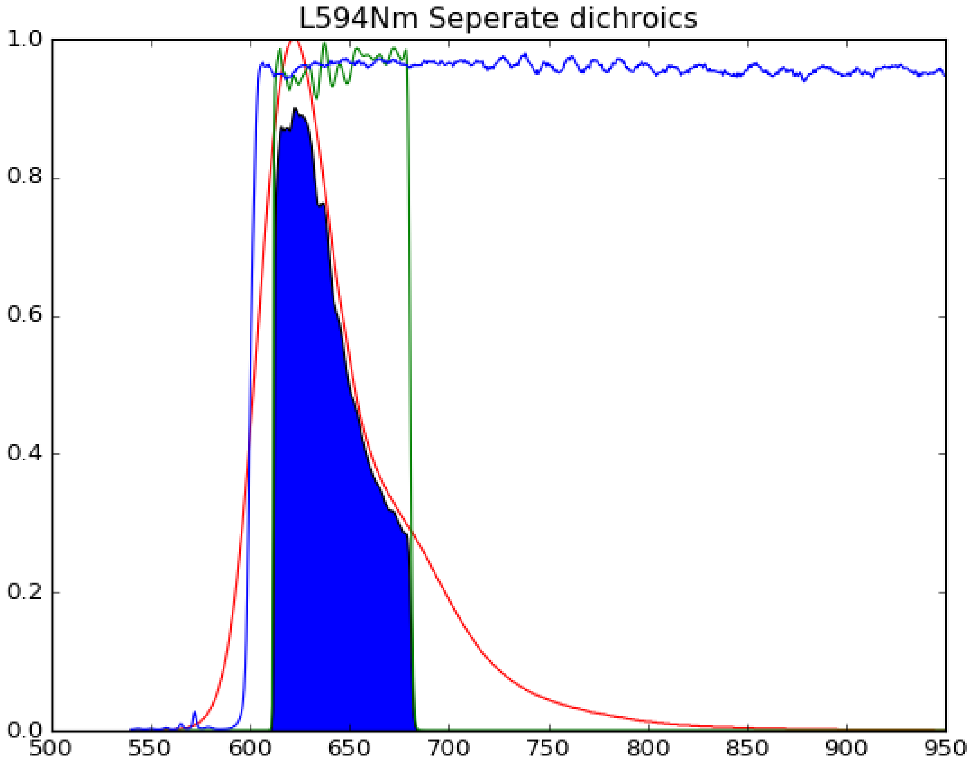
```
% matplotlib notebook
ratios = []
ch_labels = []

for l, f_old, f_new, dy in zip(laser_list, fc_list, multi_fc_list, dye_list):
    d, ch, sig_new = signalFromDyeXInChannelY(l, f_new, dy, objective, camera)
    d, ch, sig_old = signalFromDyeXInChannelY(l, f_old, dy, objective, camera)

    ratios.append(sig_new/sig_old)
    ch_labels.append(ch)
#     print(ch)
    #print(sig_old)
    #print(sig_new)
    #print('Fraction = {}'.format(sig_new/sig_old))
    #fig = plt.figure();
    #plt.title('Old cubes, ' + ch + ' channel')
    #f_old.displaySpectra()
    #fig2 = plt.figure()
    #plt.title('Multiedge cube, ' + ch + ' channel')
    #f_new.displaySpectra()
    ol1 = show_dye_emission_enclosed_by_filters(dy, f_old, objective, camera, title='Se
parate dichroics')
    ol2 = show_dye_emission_enclosed_by_filters(dy, f_new, objective, camera, title='Mu
ltiedge dichroic')


fig3 = plt.figure();
plt.bar([1, 2, 3, 4, 5],
        ratios,
        tick_label=ch_labels,
        align='center')
plt.ylabel('Fractional signal, multiedge/separate')
```

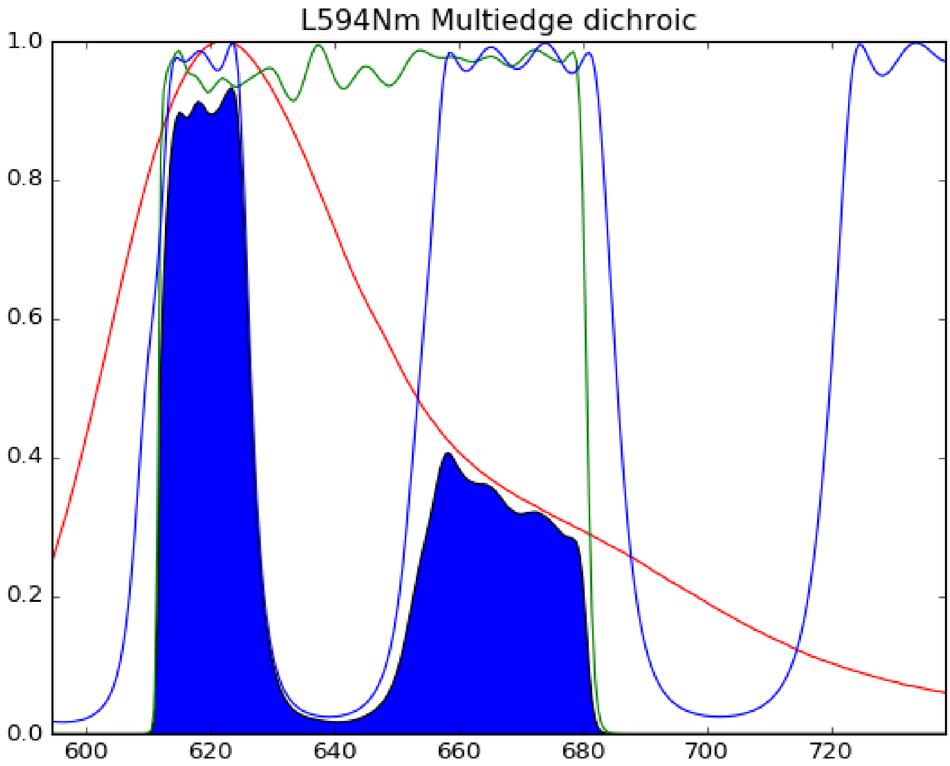

L405Nm Seperate dichroics
1.0
0.8
0.6
0.4
0.2
0.0
350
400
450
500
550
600

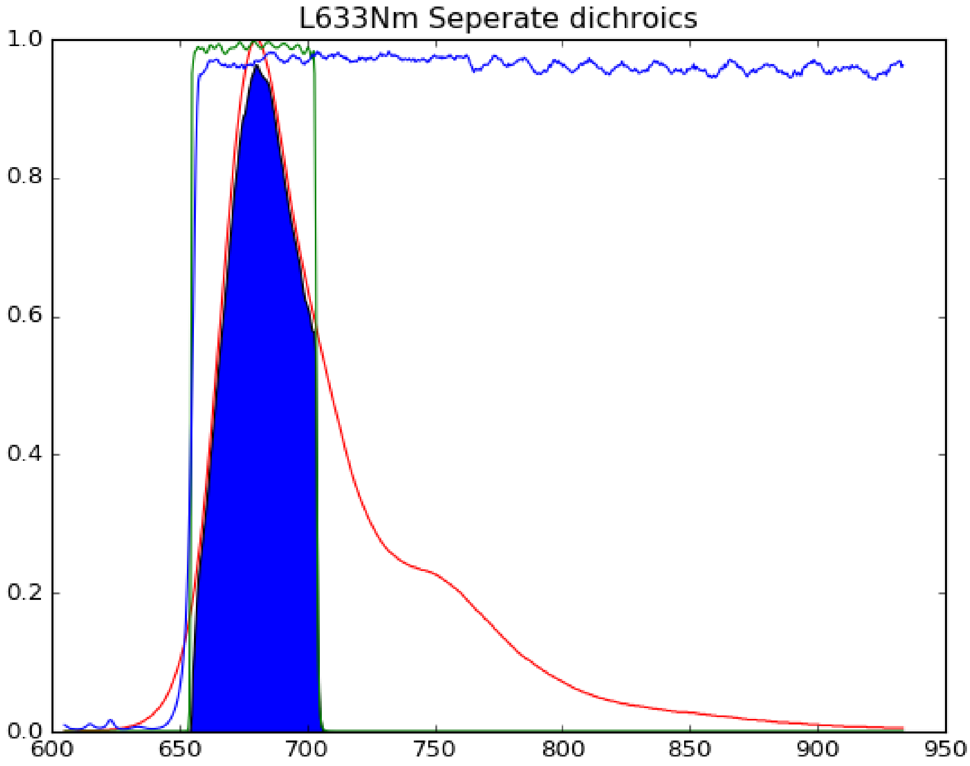

L405Nm Multiedge dichroic
1.0
0.8
0.6
0.4
0.2
0.0
350
400
450
500
550
600

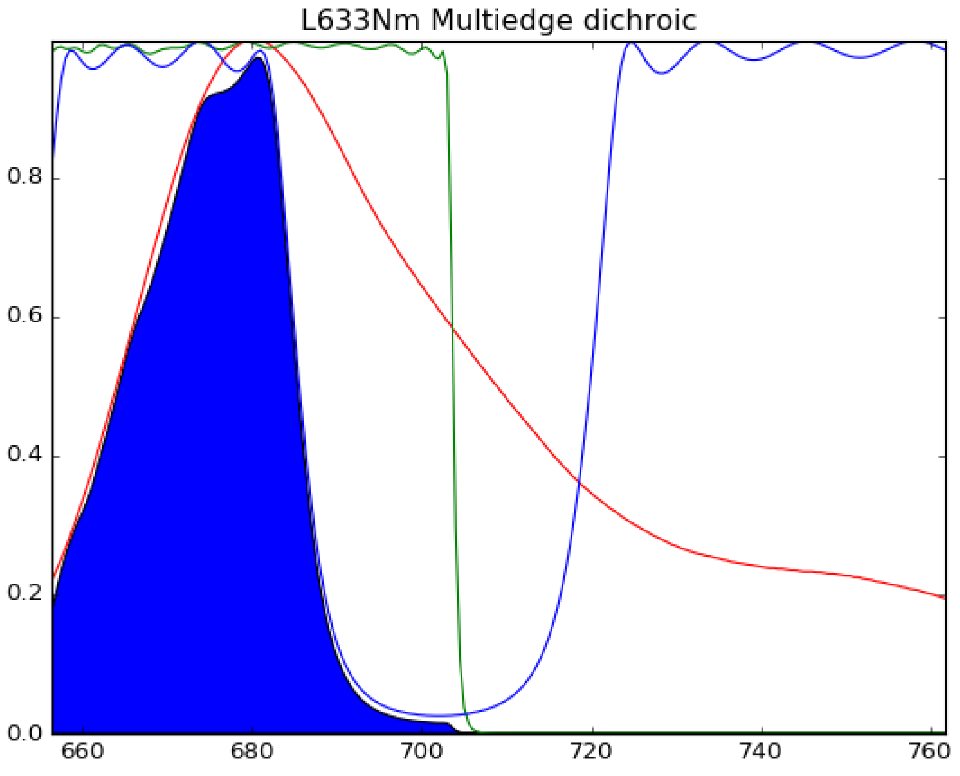

L532Nm Seperate dichroics
1.0
0.8
0.6
0.4
0.2
0.0
450
500
550
600
650
700
750
800
850

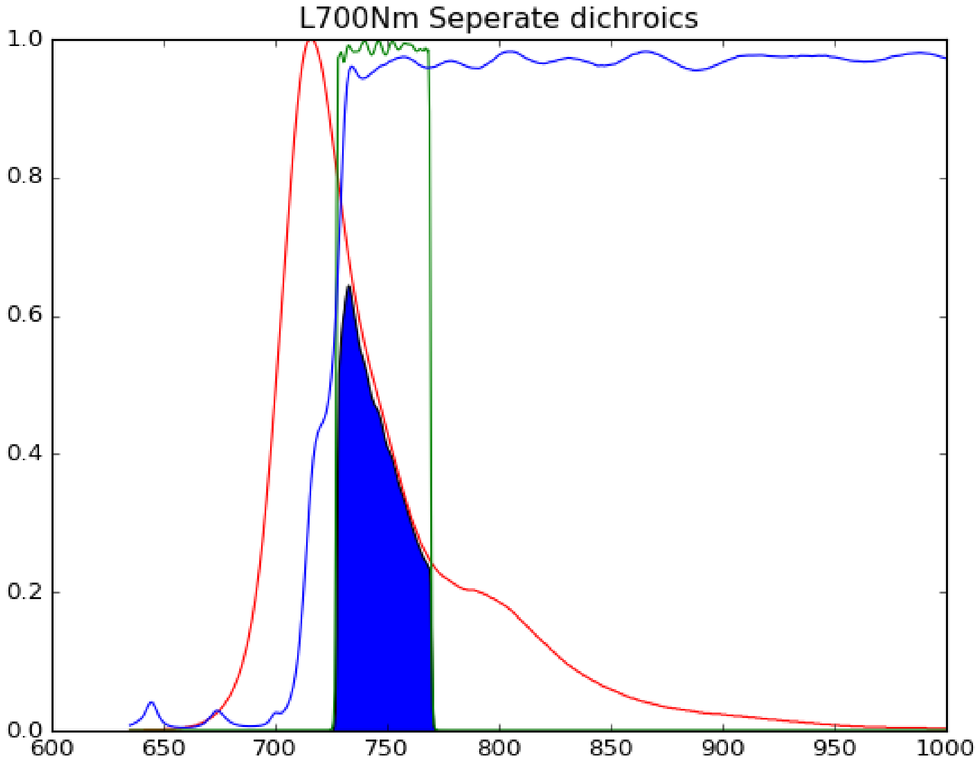

L532Nm Multiedge dichroic
1.0
0.8
0.6
0.4
0.2
0.0
450
500
550
600
650
700
750
800
850

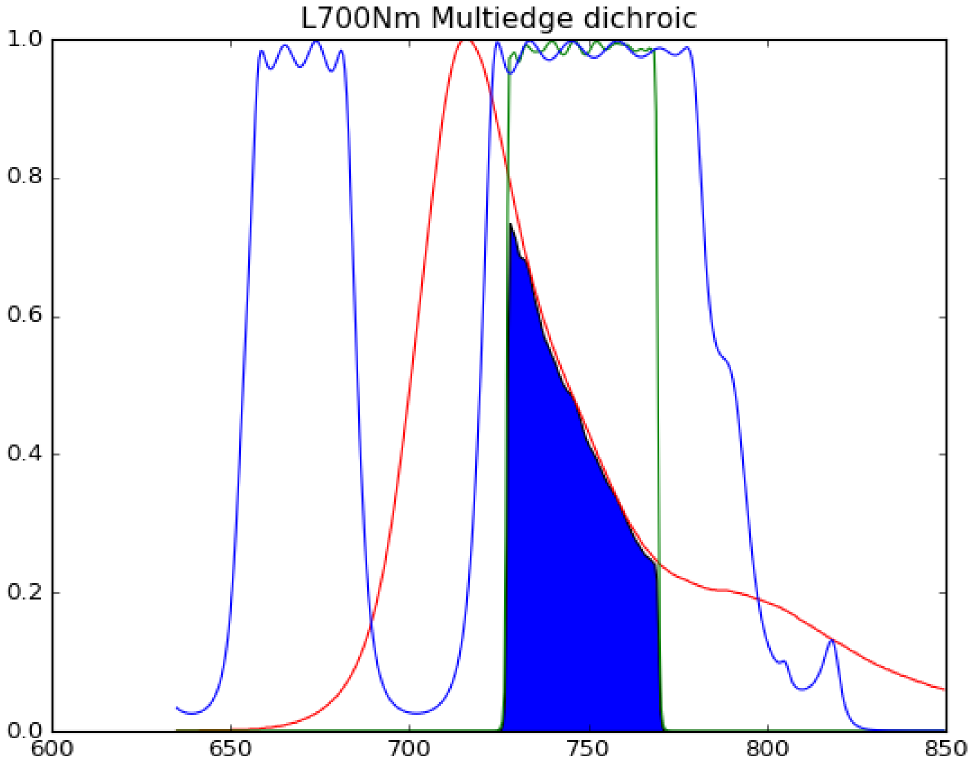

L594Nm Seperate dichroics
1.0
0.8
0.6
0.4
0.2
0.0
500
550
600
650
700
750
800
850
900
950

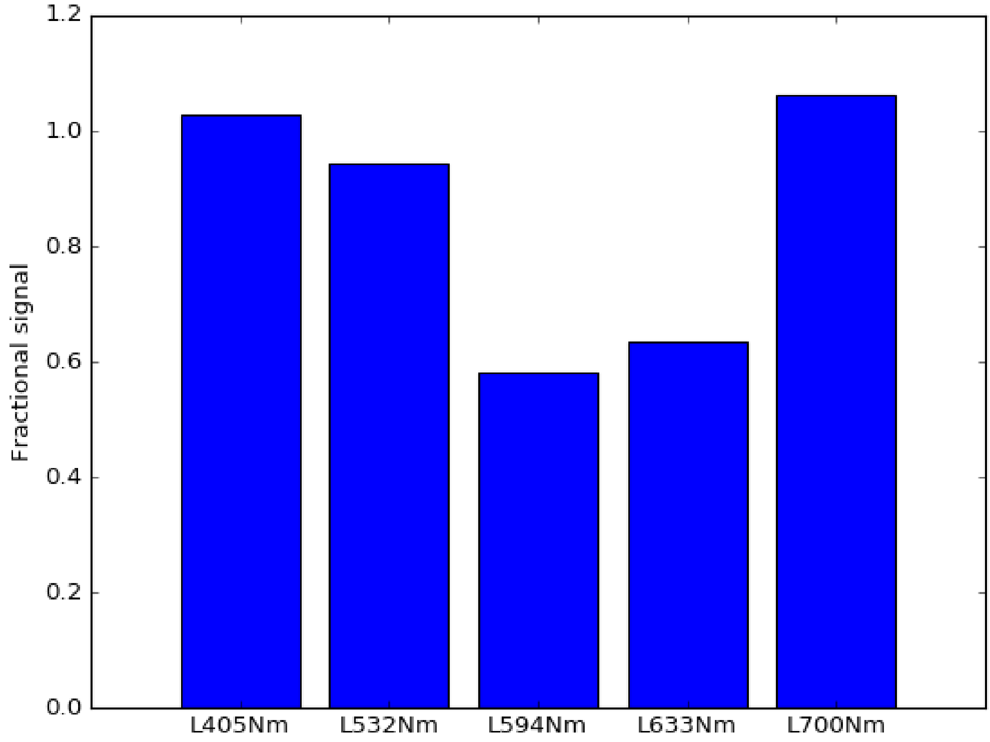

L594Nm Multiedge dichroic
1.0
0.8
0.6
0.4
0.2
0.0
600
620
640
660
680
700
720


L633Nm Seperate dichroics
1.0
0.8
0.6
0.4
0.2
0.0
600
650
700
750
800
850
900
950


L633Nm Multiedge dichroic
0.8
0.6
0.4
0.2
0.0
660
680
700
720
740
760


L700Nm Seperate dichroics
1.0
0.8
0.6
0.4
0.2
0.0
600
650
700
750
800
850
900
950
1000


L700Nm Multiedge dichroic
1.0
0.8
0.6
0.4
0.2
0.0
600
650
700
750
800
850



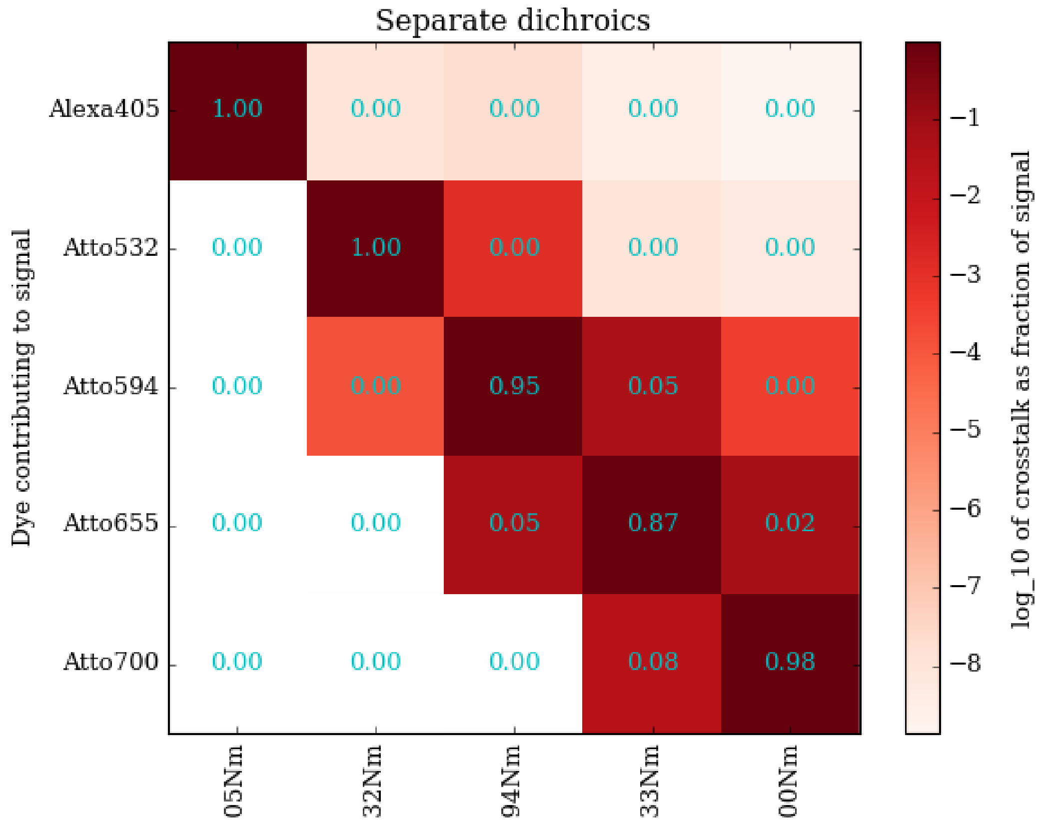

```
Out[6]:

<matplotlib.text.Text at 0x2b3a90afdd8>
```

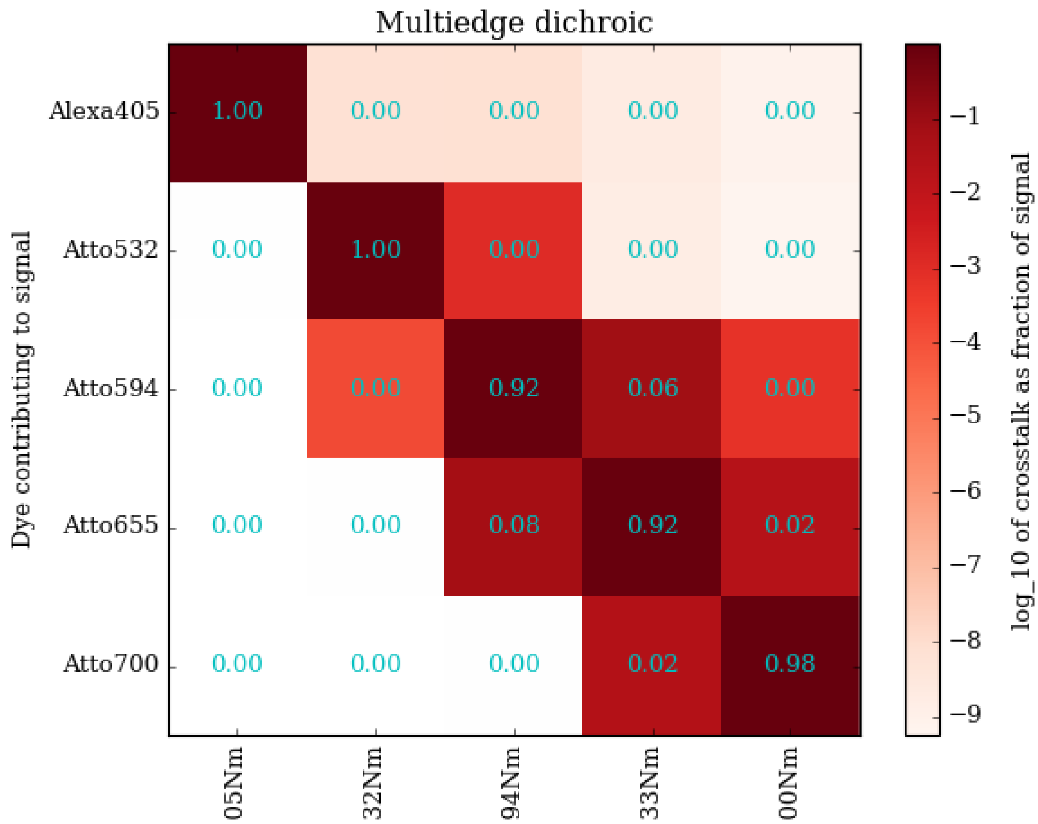
Crosstalk comparison

Consider next the crosstalk between channels:

In [7]:

```
displayCrosstalkPlot(laser_list,
                     fc_list,
                     dye_list,
                     objective, camera)

plt.title('Separate dichroics')

displayCrosstalkPlot(laser_list,
                     multi_fc_list,
                     dye_list,
                     objective, camera)

plt.title('Multiedge dichroic')
```





Out[7]:

```
<matplotlib.text.Text at 0x2b3aaeb62b0>
```

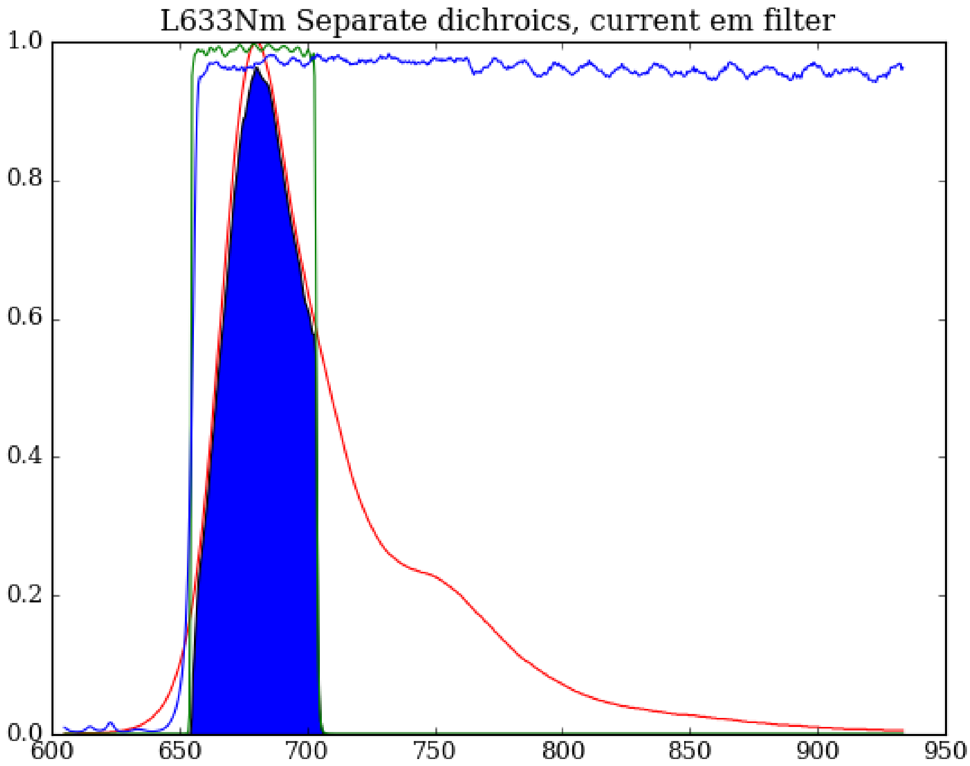
Alternative emission filters

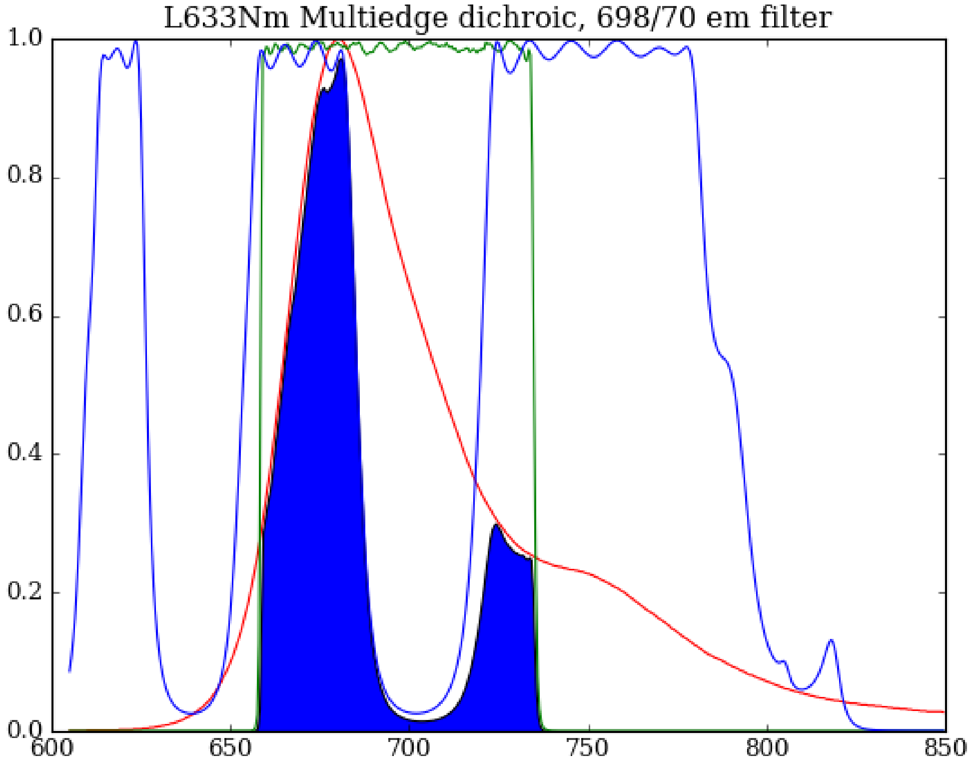
Can we recover signal by increasing the emission cut-off wavelength in 594 and 633 channels?

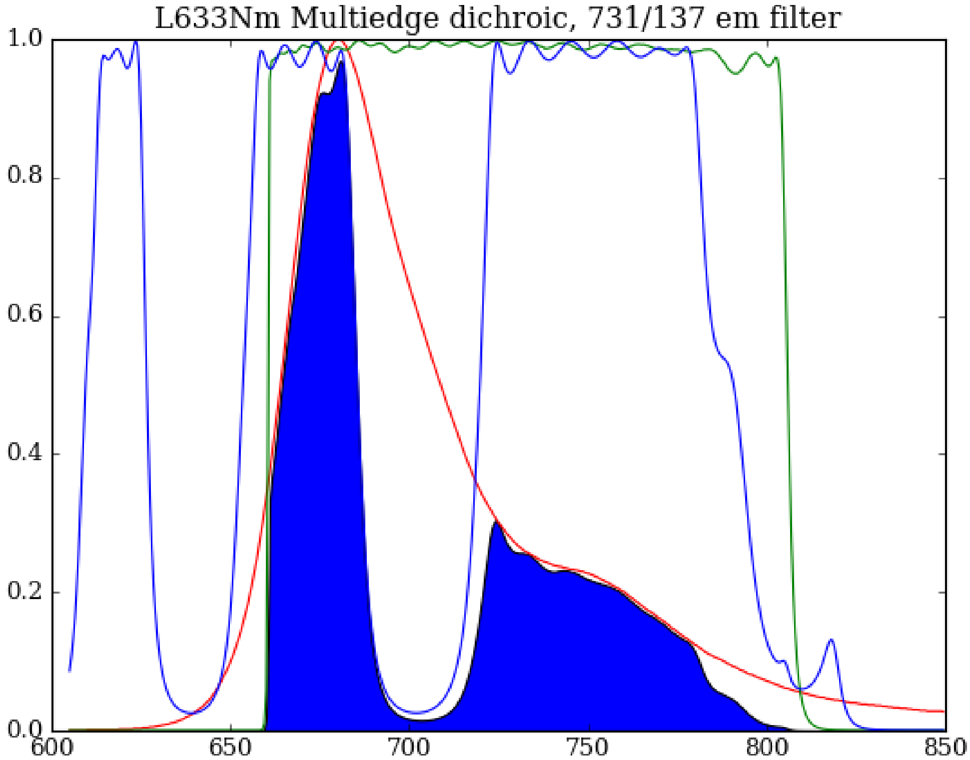
In [20]:

```
ratios_maxem = ratios

fc633multi2 = FilterCube(channel = 'L633Nm',
                   excitationFilter = ( 'FF01-640_14', os.path.join(filtersPath, 'FF01-
640_14_spectrum.txt') ),
                   dichroicFilter = ( 'Chroma multiedge', os.path.join(filtersPath, 'Ch
roma ZT405-532-594-640-701rpc.txt') ),
                   emissionFilter = ( 'FF01-698_70', os.path.join(filtersPath, 'FF01-69
8_70_Spectrum.txt') ) )

fc633multi3 = FilterCube(channel = 'L633Nm',
                   excitationFilter = ( 'FF01-640_14', os.path.join(filtersPath, 'FF01-
640_14_spectrum.txt') ),
                   dichroicFilter = ( 'Chroma multiedge', os.path.join(filtersPath, 'Ch
roma ZT405-532-594-640-701rpc.txt') ),
                   emissionFilter = ( 'FF01-731_137', os.path.join(filtersPath, 'FF01-7
31_137_Spectrum.txt') ) )

d, ch, sig_new2 = signalFromDyeXInChannelY(l633, fc633multi2, dye633, objective,
camera)
d, ch, sig_new3 = signalFromDyeXInChannelY(l633, fc633multi3, dye633, objective,
camera)
d, ch, sig_old = signalFromDyeXInChannelY(l633, fc633, dye633, objective, camera)

ol1 = show_dye_emission_enclosed_by_filters(dye633, fc633, objective, camera, title='Se
parate dichroics, current em filter')
ol2 = show_dye_emission_enclosed_by_filters(dye633, fc633multi2, objective, camera, tit
le='Multiedge dichroic, 698/70 em filter')
ol3 = show_dye_emission_enclosed_by_filters(dye633, fc633multi3, objective, camera, tit
le='Multiedge dichroic, 731/137 em filter')

print('633 signal ratio, 698/70 = {:0.3f}'.format(sig_new2/sig_old))
print('633 signal ratio, 731/137 = {:0.3f}'.format(sig_new3/sig_old))

ratios_maxem[3] = sig_new3/sig_old

fc594multi2 = FilterCube(channel = 'L594Nm',
                   excitationFilter = ( 'FF01-591_6', os.path.join(filtersPath, 'FF01-5
91_6_Spectrum.txt') ),
                   dichroicFilter = ( 'Chroma multiedge', os.path.join(filtersPath, 'Ch
roma ZT405-532-594-640-701rpc.txt') ),
                   emissionFilter = ( 'FF02-650_100', os.path.join(filtersPath, 'FF02-6
50_100_Spectrum.txt') ) )

fc594multi3 = FilterCube(channel = 'L594Nm',
                   excitationFilter = ( 'FF01-591_6', os.path.join(filtersPath, 'FF01-5
91_6_Spectrum.txt') ),
                   dichroicFilter = ( 'Chroma multiedge', os.path.join(filtersPath, 'Ch
roma ZT405-532-594-640-701rpc.txt') ),
                   emissionFilter = ( 'FF02-641_75', os.path.join(filtersPath, 'FF02-64
1_75_Spectrum.txt') ) )

fc594multi4 = FilterCube(channel = 'L594Nm',
```

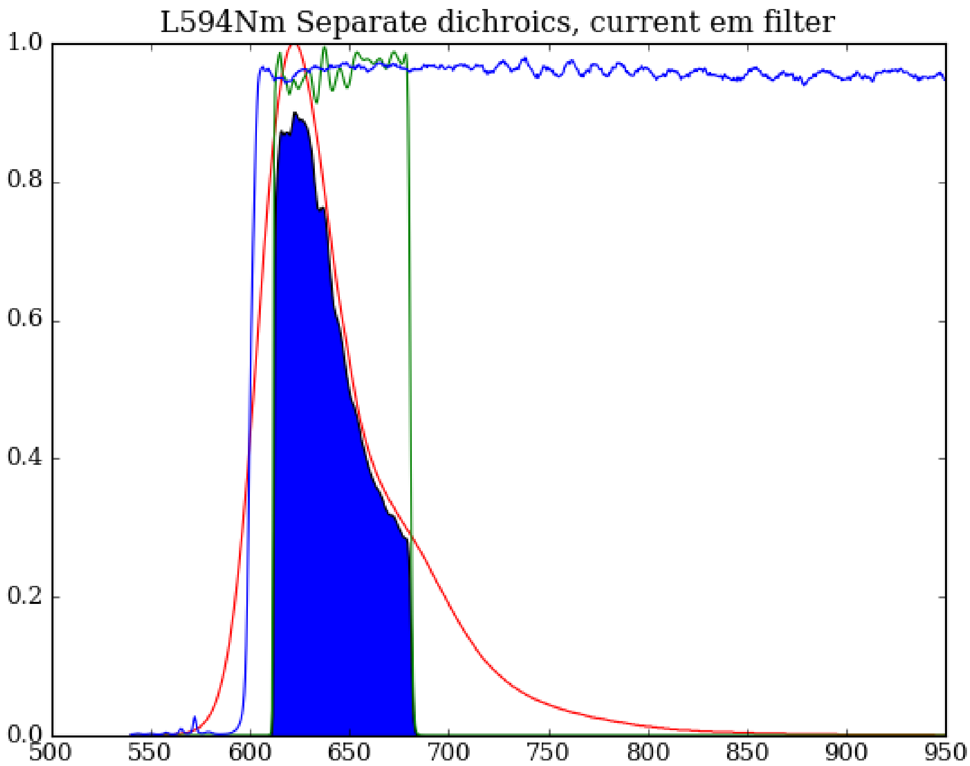
```
                   excitationFilter = ( 'FF01-591_6', os.path.join(filtersPath, 'FF01-5
91_6_Spectrum.txt') ),
                   dichroicFilter = ( 'Chroma multiedge', os.path.join(filtersPath, 'Ch
roma ZT405-532-594-640-701rpc.txt') ),
                   emissionFilter = ( 'BLP01-594R', os.path.join(filtersPath, 'BLP01-59
4R_Spectrum.txt') ) )


d, ch, sig_new2 = signalFromDyeXInChannelY(l594, fc594multi2, dye594, objective,
camera)
d, ch, sig_new3 = signalFromDyeXInChannelY(l594, fc594multi3, dye594, objective,
camera)
d, ch, sig_new4 = signalFromDyeXInChannelY(l594, fc594multi4, dye594, objective,
camera)
d, ch, sig_old = signalFromDyeXInChannelY(l594, fc594, dye594, objective, camera)

ol1 = show_dye_emission_enclosed_by_filters(dye594, fc594, objective, camera, title='Se
parate dichroics, current em filter')
ol2 = show_dye_emission_enclosed_by_filters(dye594, fc594multi2, objective, camera, tit
le='Multiedge dichroic, 650/100 em filter')
ol3 = show_dye_emission_enclosed_by_filters(dye594, fc594multi3, objective, camera, tit
le='Multiedge dichroic, 641/75 em filter')
ol4 = show_dye_emission_enclosed_by_filters(dye594, fc594multi4, objective, camera, tit
le='Multiedge dichroic, 594LP em filter')

print('594 signal ratio, 650/100 = {:0.3f}'.format(sig_new2/sig_old))
print('594 signal ratio, 641/75 = {:0.3f}'.format(sig_new3/sig_old))
print('594 signal ratio, 594LP = {:0.3f}'.format(sig_new4/sig_old))


ratios_maxem[2] = sig_new4/sig_old

fig4 = plt.figure();
plt.bar([1, 2, 3, 4, 5],
        ratios_maxem,
        tick_label=ch_labels,
        align='center')
plt.ylabel('Fractional signal, multiedge/separate')
```

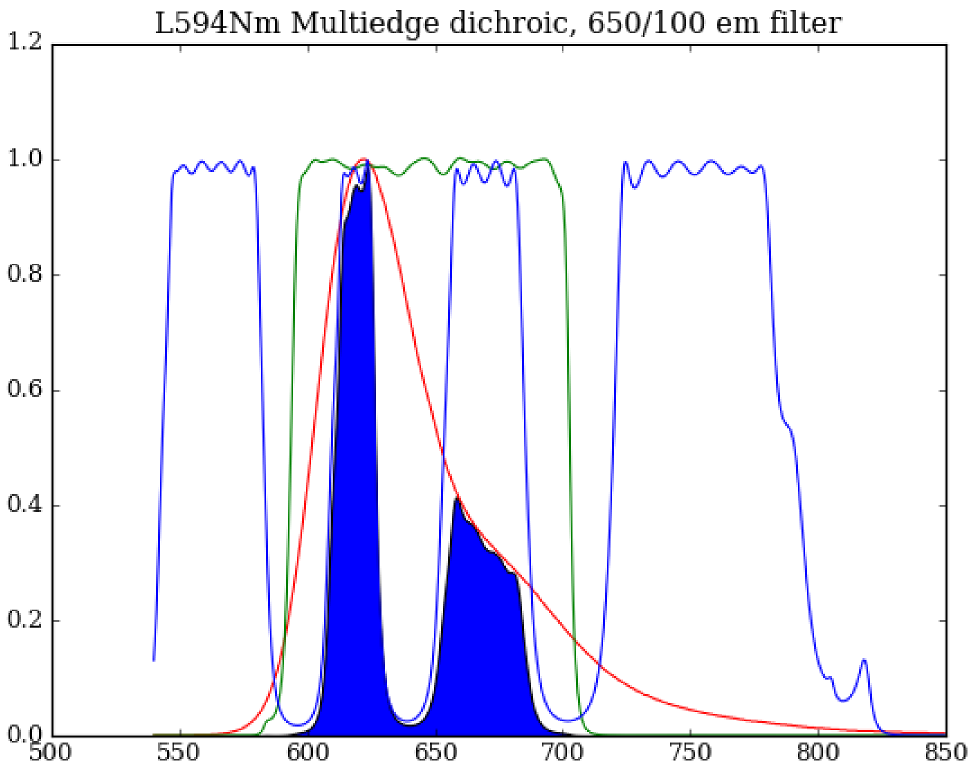

L633Nm Separate dichroics, current em filter
1.0
0.8
0.6
0.4
0.2
0.0
600
650
700
750
800
850
900
950

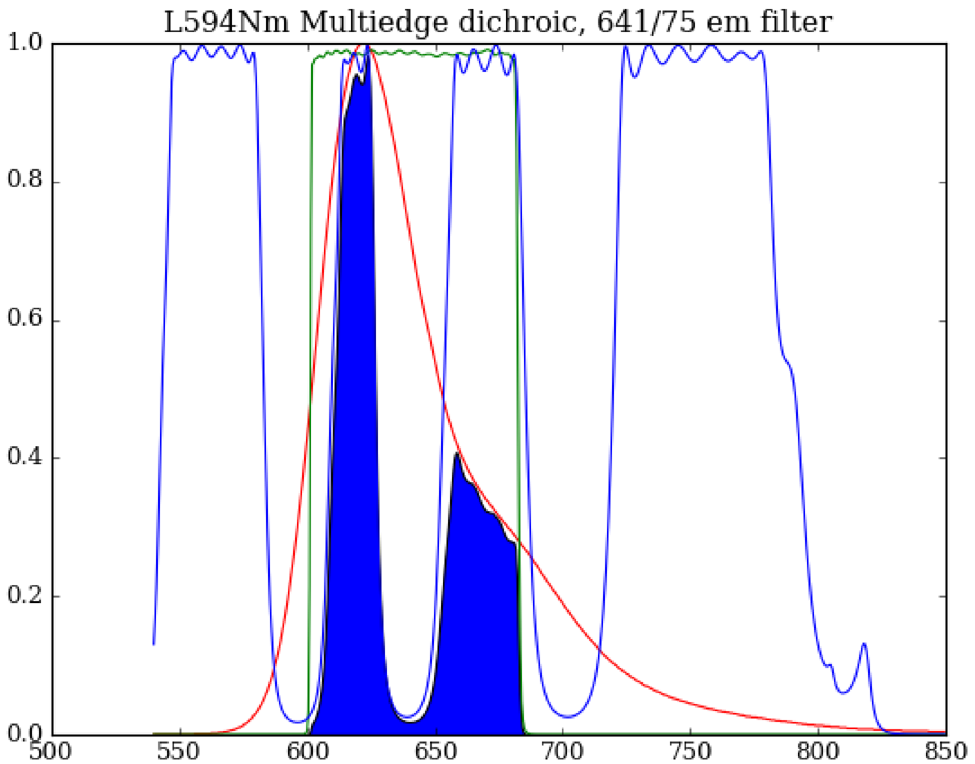

L633Nm Multiedge dichroic, 698/70 em filter
1.0
0.8
0.6
0.4
0.2
0.0
600
650
700
750
800
850



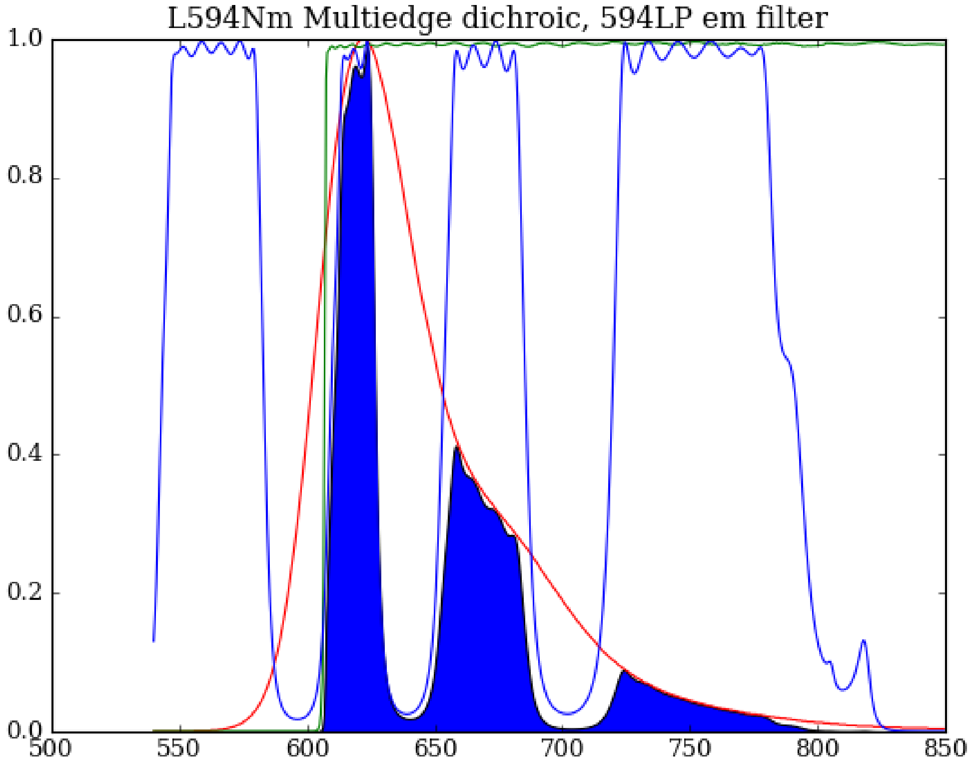
633 signal ratio, 698/70 = 0.731
633 signal ratio, 731/137 = 0.913



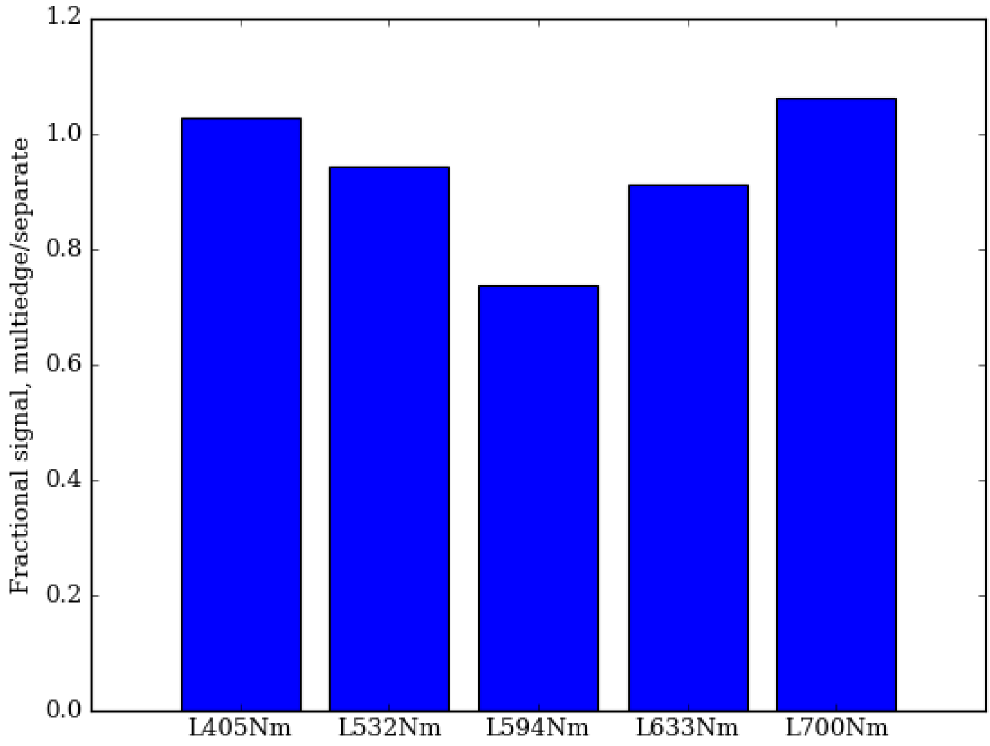





```
C:\Users\d.kelly\AppData\Local\Continuum\Anaconda3\lib\site-packages\matpl
otlib\pyplot.py:516: RuntimeWarning: More than 20 figures have been opene
d. Figures created through the pyplot interface (`matplotlib.pyplot.figure
`) are retained until explicitly closed and may consume too much memory.
 (To control this warning, see the rcParam `figure.max_open_warning`).
  max_open_warning, RuntimeWarning)
```




```
594 signal ratio, 650/100 = 0.685
594 signal ratio, 641/75 = 0.658
594 signal ratio, 594LP = 0.737
```




```
Out[20]:
<matplotlib.text.Text at 0x2b3ad0e1588>
```