

# Technical specifications of the epifluorescent imaging equipment

|              | Options               | Britemac + Limelight | Macred         | Discovery      |
|--------------|-----------------------|----------------------|----------------|----------------|
| Objectives   | Air                   | 2.5, 5, 10, 20       | 2.5, 5, 10, 20 | 2.5, 5, 10, 20 |
|              | Oil                   | 40, 63, 100          | 40, 63, 100    | 40, 63, 100    |
| Fluorophores |                       | B, G, R, Y, FR       | B, G, R        | B, G, R        |
| Filtersets   | See list on next page | Chroma3 + Sedat quad | Chroma3        | Chroma3        |
| Light Source | Trans.                | White light          | White light    | White LED      |
|              | Fluo.                 | Exfo                 | ExFo           | ExFo           |
| Camera       |                       | Mono                 | Mono           | Mono +         |
|              |                       |                      |                | colour         |
| Techniques   | Colourimetric         | Yes                  | No             | Yes            |
|              | Multi channel         | Yes                  | Yes            | Yes            |
|              | Z-Stack               | No                   | No             | No             |
|              | Tiling                | No                   | No             | No             |

|              | Options       | Granny         | Pinklady       | Calvados          | Santana       |
|--------------|---------------|----------------|----------------|-------------------|---------------|
| Objectives   | Air           | 10             | 5, 10, 20, 40  | 5, 10, 20, 40     | 5, 10, 20, 40 |
|              | Oil           | 63, 100        |                | 40, 63, 100       | 63, 100       |
| Fluorophores |               | B, G, R, Y, FR | B, G, R, Y, FR | B, C, G, R, Y, FR |               |
| Light Source | Trans.        |                | White light    | White light       | White light   |
|              | Fluo.         | Lumen Fluo     | ExFo           | LED               | ExFo          |
| Camera       |               | Mono           | Mono           | Mono              | Mono          |
| Techniques   |               | Deconvolution  | Tiling         | Live imaging      | Live imaging  |
| Techniques   | Colourimetric | No             | Yes            | No                | No            |
|              | Multi channel | Yes            | Yes            | Yes               | Yes           |
|              | Z-Stack       | Yes            | No             | Yes               | Yes           |
|              | Tiling        | No             | Yes            | Yes               | Yes           |

| Fluorophore | Excitation   | Emission    | Dye example             |
|-------------|--------------|-------------|-------------------------|
| Blue        | 350 - 410nm  | 380 - 440nm | DAPI, Hoechst, AF405    |
| Green       | 480 - 505 nm | 510 - 540nm | AF488, FITC, CY2, GFP   |
| Yellow      | 514 - 540nm  | 530 - 550m, | eYFP, Cy3, TRITC, AF543 |
| Red         | 560 - 600nm  | 580 - 620nm | mCherry                 |
| Far Red     | 630 - 670nm  | 650 - 720nm | Cy5, AF647, Atto 650    |

Other fluorochromes can be used with these filter sets, so long as they fall between the excitation and emission values of the filters. A fuller list can be found in Common Fluorophores section on the website or online, where the fluorophore name can be entered and the excitation/emission spectra can be viewed.

<https://searchlight.semrock.com/>

Check compatibility of the combination of dyes and fluorescent proteins chosen with our systems and ensure there is no spectral overlap using the spectra viewer here:

<https://www.thermofisher.com/uk/en/home/life-science/cell-analysis/labeling-chemistry/fluorescence-spectraviewer.html>

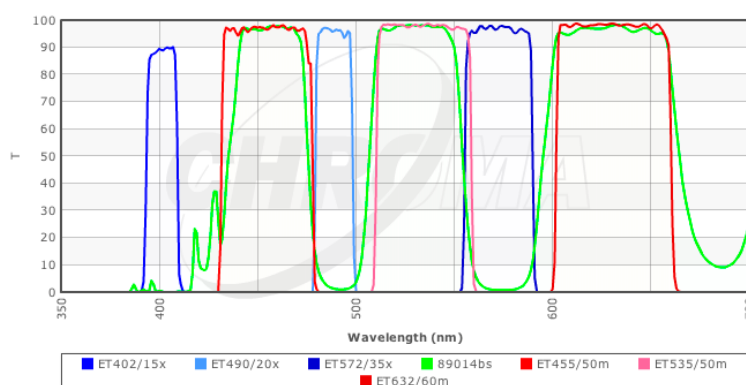
## Microscopes: Discovery, Macred, Limelight, Britemac

### Filter Set Name:

Chroma 3 (AKA) 89014 DAPI/FITC/Texas Red ET

<https://www.chroma.com/products/sets/89014-et-dapi-fitc-texas-red>

| Excitation | Emission  | Suitable dyes                                |
|------------|-----------|----------------------------------------------|
| 395 - 410  | 430 - 480 | DAPI, Alexa 405, Hoechst 34580, Pacific Blue |
| 480 - 500  | 510 - 560 | FITC, Alexa 488, GFP, Cy2, MitoTracker Green |
| 554 - 589  | 602 - 662 | TxRd, Alexa568, Alexa 594, RFP, mCherry      |



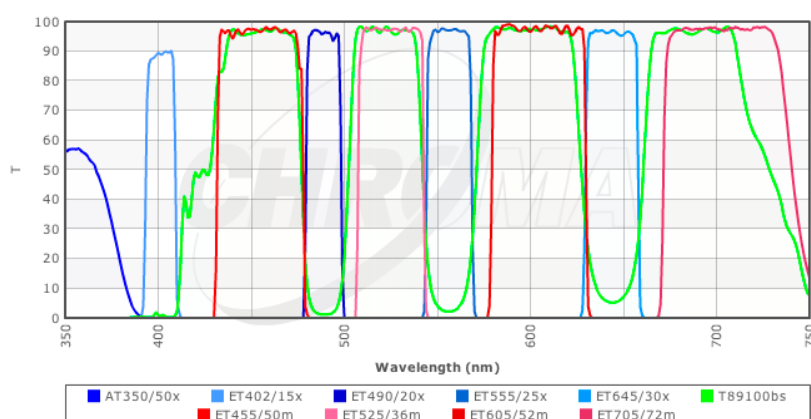
## Microscopes: Britemac, Limelight

### Filter Set Name:

Chroma 4 AKA 89000 Sedat Quad ET

<https://www.chroma.com/products/sets/89000-et-sedat-quad>

| Excitation | Emission  | Suitable dyes                                |
|------------|-----------|----------------------------------------------|
| 395 - 410  | 430 - 480 | DAPI, Alexa 405, Hoechst 34580, Pacific Blue |
| 480 - 500  | 507 - 543 | FITC, Alexa 488, GFP, Cy2, MitoTracker Green |
| 543 - 568  | 579 - 631 | TRITC, DsRed, Cy3, MitoTracker Orange        |
| 630 - 660  | 669 - 741 | Cy5, Alexa647, BODIPY650                     |



## Microscope: Calvados

Semrock Briteline LED Quad Pinkel DAPI/FITC/TRITC/Cy5

<https://www.semrock.com/SetDetails.aspx?id=3532>

| Name in Micro-Manager | Excitation | Emission | Suitable dyes                                |
|-----------------------|------------|----------|----------------------------------------------|
| DAPI Quad             | 392/23     | 414-450  | DAPI, Alexa 405, Hoechst 34580,              |
| GFP Quad              | 474/27     | 500-530  | FITC, Alexa 488, GFP, Cy2, MitoTracker Green |
| RFP Quad              | 554/23     | 580-611  | TRITC, tagRed, Cy3, MitoTracker Orange       |
| Alexa647 Quad         | 635/18     | 661-800  | Cy5, Alexa647, SiR-DNA                       |

Semrock single cubes GFP and RFP ([www.semrock.com](http://www.semrock.com))

| Name in Micro-Manager | Excitation | Emission | Semrock Part No |
|-----------------------|------------|----------|-----------------|
| GFP Single            | 474/27     | 520/28   | GFP-A-Basic-000 |
| RFP Single            | 554/23     | 609/54   | LED-TRITC-A-000 |
| CFP                   | 434/17     | 479/40   | CFP-A-Basic-000 |
| YFP                   | 497/16     | 535/22   | YFP-A-Basic-000 |

## Microscope: Thunder (Widefield Mode)

Semrock BrightLine Quad (Sedat) LED-DAPI/FITC/TRITC/Cy5-4X4M-B

| Fluorophore | LED wavelength | Excitation | Emission | Dichroic |
|-------------|----------------|------------|----------|----------|
| DAPI        | 365            | 378/52*    | 432/36*  | 410      |
| FITC        | 470            | 474/27*    | 515/30*  | 504      |
| TRITC       | 580            | 554/23*    | 595/31*  | 582      |
| Cy5         | 635            | 635/18     | 698/70   | 669      |

Semrock BrightLine Triple (Pinkel) LED-CFP/YFP/mCherry-3X-A

| Fluorophore | LED wavelength | Excitation | Emission | Dichroic |
|-------------|----------------|------------|----------|----------|
| CFP         | 435            | 438/24     | 475      | 459      |
| YFP         | 500            | 509/22     | 543      | 526      |
| mCherry     | 580            | 578/21     | 702      | 596      |

## Nikon single band filter cubes

| Fluorophore   | Excitation | Emission | Dichroic |
|---------------|------------|----------|----------|
| DAPI          | 375/28     | 460/60   | 415      |
| GFP-B         | 470/40     | 535/50   | 500      |
| mCherry/TR HQ | 570/40     | 645/75   | 600      |

If you use these cubes in combination with the Prime BSI camera you will have additional excitation and emission filters in the optical path. The additional filters in question are indicated by a \* in the tables above.

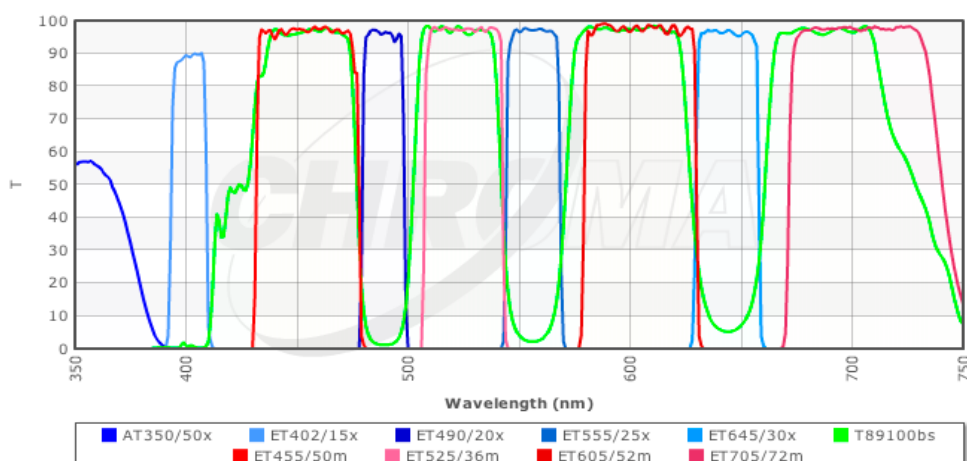
## Microscope: Santana

### Filter Set Name: (Microscope Position 1)

Chroma 4 AKA 89000 Sedat Quad ET

<https://www.chroma.com/products/sets/89000-et-sedat-quad>

| Excitation | Emission  | Suitable dyes                                |
|------------|-----------|----------------------------------------------|
| 395 - 410  | 430 - 480 | DAPI, Alexa 405, Hoechst 34580, Pacific Blue |
| 480 - 500  | 507 - 543 | FITC, Alexa 488, GFP, Cy2, MitoTracker Green |
| 543 - 568  | 579 - 631 | TRITC, DsRed, Cy3, MitoTracker Orange        |
| 630 - 660  | 669 - 741 | Cy5, Alexa647, BODIPY650                     |



### Filter Set Name: (Microscope Position 2)

Chroma 89903 - ET - BV421/BV480/AF488/AF568/AF647 Quinta Band set

<https://www.chroma.com/products/sets/89903-et-bv421-bv480-af488-af568-af647-quinta-band-set>

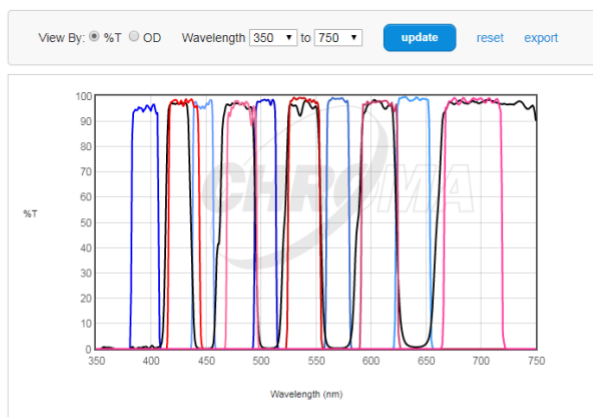
| Excitation | Emission  | Suitable dyes                 |
|------------|-----------|-------------------------------|
| 383 - 407  | 417 - 445 | Alexa 405, DAPI, Hoechst, BFP |
| 429 - 467  | 469 - 495 | CFP                           |
| 495 - 515  | 525 - 555 | Cy3, TRITC, eYFP, GFP         |
| 560 - 580  | 592 - 626 | Texas red, mCherry            |
| 625 - 655  | 665 - 715 | Cy5, Alexa647, BODIPY650      |

### 89903 - ET – BV421/BV480/AF488/AF568/AF647 Quinta Band set

Price: \$3,450.00

For use with BD Horizon Brilliant™ Violet 421 and Brilliant™ Violet 480 in 5-color fluorescence imaging scheme. Separate filter housings (such as external filter wheels) required for individual excitation and emission filters.

| Legend                              | Fluorochromes | Sets  | Parts |
|-------------------------------------|---------------|-------|-------|
| Catalog Sets                        |               |       |       |
| 89903                               |               |       |       |
| Hide Individual Filters x           |               |       |       |
| PLOT                                | FILTERS       | DATA  |       |
| <input checked="" type="checkbox"/> | ET395/25x     | ASCII |       |
| <input checked="" type="checkbox"/> | ET448/19x     | ASCII |       |
| <input checked="" type="checkbox"/> | ET505/20x     | ASCII |       |
| <input checked="" type="checkbox"/> | ET570/20x     | ASCII |       |
| <input checked="" type="checkbox"/> | ET640/30x     | ASCII |       |
| <input checked="" type="checkbox"/> | 89903bs       | ASCII |       |
| <input checked="" type="checkbox"/> | ET431/28m     | ASCII |       |
| <input checked="" type="checkbox"/> | ET482/27m     | ASCII |       |
| <input checked="" type="checkbox"/> | ET540/30m     | ASCII |       |
| <input checked="" type="checkbox"/> | ET609/34m     | ASCII |       |
| <input checked="" type="checkbox"/> | ET690/50m     | ASCII |       |



All transmission and blocking (OD) data are actual, measured spectra of representative production lots. Spectra varies slightly from lot to lot. Optical density values in excess of 6 may appear noisy because such evaluations push the resolution limit of low light level measurements.

## Microscope: Pink Lady

<https://micro.magnet.fsu.edu/primer/techniques/fluorescence/fluorotable3.html>

| Single filter cube | Excitation  | Emission  | Suitable dyes          |
|--------------------|-------------|-----------|------------------------|
| DAPI               | 352 - 402   | 417 - 477 | DAPI,                  |
| Aqua               | 426 - 450   | 502 - 538 | CFP                    |
| Green              | 457 - 487   | 502 - 538 | FITC, AF488, GFP, Cy2, |
| Gold               | 488 - 512   | 532 - 554 | YFP                    |
| Orange             | 532 - 554   | 570 - 613 | TRITC, Cy3, AF555      |
| Red                | 542 - 582   | 603 - 678 | Texas Red, AF594       |
| U-MF2              | Brightfield |           |                        |

## Mesoscopes

### Microscope: Nikon Tissue Culture

| Name | Excitation | Emission | Suitable dyes        |
|------|------------|----------|----------------------|
| UV   | 340-380    | 435-485  | DAPI, BFP            |
| B    | 460-500    | 510-560  | GFP, FITC, Alexa 488 |
| G    | 572/23     | 630/60   |                      |
| CFP  | 436/20     | 480/40   |                      |
| YFP  | 500/20     | 535/30   |                      |

**Microscope: Nikon Macroscope (N.B. can share filters with Tissue culture if needed. Please ask facility staff if needed)**

| <b>Name</b> | <b>Excitation</b> | <b>Emission</b> | <b>Suitable dyes</b> |
|-------------|-------------------|-----------------|----------------------|
| GFP         | 460-500           | 510LP           | GFP, FITC            |
| G-2A        | 510-560           | 590LP           | TRITC, RFP           |

In addition for Calvados there is:

Semrock Dual band GFP/DsRed filter set (Requires Cairn Optosplit to separate and image both wavelengths simultaneously)

\*Not currently configured in Micro-Manager-ask facility staff if required

| <b>Excitation</b>                                          | <b>Emission</b> | <b>Semrock Part No</b> |
|------------------------------------------------------------|-----------------|------------------------|
| 468/553 (Optimised for GFP – RFP simultaneous acquisition) | 512/630         | GFP/DsRed-2X2M-C-000   |