

Nikon Microscope Parameters (Scope I)

Excitation Filters: anticlockwise, Lambda Wheel A

- Filter 1 (Position 0): 420/20 nm -> 410 -430 nm CFP excitation
- Filter 2 (Position 1): 546/11 nm mOrange1 excitation
- Filter 3 (Position 2): 590/40 nm for Red Photoactivation (previously 340 nm Fura2 excitation).
- Filter 4 (Position 3): 660/20 nm for Red Photo-deactivation (previously 380 nm Fura2 excitation 2, broken though).
- Filter 5 (Position 4): 495/10x nm YFP excitation
- Filter 6 (Position 5): 560/40 nm mCherry1 excitation
- Filter 7 (Position 6): 515/10x nm mOrange2 excitation
- Filter 8 (Position 7): 580/10 nm mCherry2 excitation
- Filter 9 (Position 8): 465/30 nm blue activation
- Filter 10 (Position 9): Open (future 760/20 nm red de-activation)

Emission Filters: anticlockwise, Lambda Wheel B

- Filter 1 (Position 0): 480/40 nm CFP emission
- Filter 2 (Position 1): 535/25 nm YFP emission
- Filter 3 (Position 2): 535/40 nm Fura2 emission
- Filter 4 (Position 3): 575/20 nm Orange2 emission
- Filter 5 (Position 4): 630/20 nm Cherry1 emission
- Filter 6 (Position 5): 650/100 nm Cherry2 emission
- Filter 7 (Position 6): Closed
- Filter 8 (Position 7): Closed
- Filter 9 (Position 8): Closed
- Filter 10 (Position 9): Open

Dichroic Mirrors:

Position 1: Analysis (DIC) - ANALY

- Position 2: 455 dextru (for CFP and C/Y FRET) – CFPHQ
- Position 3: 510dclp (for YFP) – G2B
- Position 4: 560 dextr (for mOrange2 and O/C FRET) - YFPHQ
- Position 5: GFP/FITC (full cube) –GFP-L
- Position 6: RFP/Tritc (full cube) - TxRed
- Backup Dichroic Mirror: 595dclp (for mCherry)

For photo-activation experiments, we switch either position 3 or position 6 to an enhanced silver mirror. We switch back to the original dichroic mirrors right after the experiment.

The arrows of filters should all facing the main body of the scope or dichroic mirror

Nikon Microscope Parameters (Scope II)

(Setting underscored if different from scope I)

Excitation Filters: anticlockwise, Lambda Wheel A

- Filter 1 (Position 0): 420/40 nm -> 400 -440 nm CFP excitation
- Filter 2 (Position 1): open
- Filter 3 (Position 2): open
- Filter 4 (Position 3): 660/20 nm for Red Photo-deactivation.
- Filter 5 (Position 4): 495/10x nm YFP excitation
- Filter 6 (Position 5): open
- Filter 7 (Position 6): 515/10x nm mOrange2 excitation
- Filter 8 (Position 7): 580/20 nm mCherry2 excitation
- Filter 9 (Position 8): 470/30 nm blue activation
- Filter 10 (Position 9): 760/20 nm red de-activation

Emission Filters: anticlockwise, Lambda Wheel B

- Filter 1 (Position 0): 480/40 nm CFP emission
- Filter 2 (Position 1): 535/30 nm YFP/Fura2 emission
- Filter 3 (Position 2): *open*
- Filter 4 (Position 3): 575/20 nm Orange2 emission
- Filter 5 (Position 4): 630/20 nm Cherry1 emission
- Filter 6 (Position 5): 650/100 nm Cherry2 emission
- Filter 7 (Position 6): Closed
- Filter 8 (Position 7): Closed
- Filter 9 (Position 8): Closed
- Filter 10 (Position 9): Open

Dichroic Mirrors:

- Position 1: Analysis (DIC) - ANALY
- Position 2: 455 dexru (for CFP and C/Y FRET) – 455LP (CFPHQ)
- Position 3: 595dclp (for mCherry) – 595LP
- Position 4: 560 dcxr (for mOrange2 and O/C FRET) – 560LP (YFPHQ)
- Position 5: GFP/FITC (full cube) –GFP-L - 49002
- Position 6: RFP/Trite (full cube) – TxRed - 49008
- Backup Dichroic Mirror: silver enhance mirror reflecting > 360 nm (2x)

Neutrodensity Filters: (Excitation ND Filter Wheel “C”

- POS1 (F2) – 2.0 – 1% Excitation
- POS2 (F3) – 1.0 – 10% Excitation
- POS3 (F4) – 0.5 – 32%
- POS4 (F5) – Open
- POS5 (F10) – Open

For photo-activation experiments, we switch either position 3 or position 6 to the silver enhanced mirror. We switch back to the original dichroic mirrors right after the experiment.

**The arrows of filters should all facing the main body of the scope or dichroic mirror
(Updated on 8/5/2014)**