**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 dcxru (for CFP and C/Y FRET) – CFPHQ

Position 3: 510dclp (for YFP) – G2B

Position 4: 560 dcxr (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 dcxru (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/Tritc (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)**