CIAN OMX SIM INSTRUCTIONS
version 2.1 September 2014

Hardware overview:
Applied Precision/GE Deltavision OMX V4 with two EM-CCD cameras (Photometrics *Evolve*), Blaze upgrade, TIRF module, DLM (Monet) module, environmental control option

Light paths:

- Transmitted light (LED illumination):
  - Bright field
  - DIC
- Wide-field fluorescence microscopy (conventional mode, solid state illumination):
  - 390/18 (= 381-399nm) 55mW (e.g. DAPI).
  - 438/24 (= 426-450nm) 85mW (e.g. CFP).
  - 475/28 (= 461-489nm) 54mW (e.g. GFP)
  - 513/17 (= 505-515nm) 22mW (e.g. YFP).
  - 575/25 (= 563-588nm) 89mW (e.g. RFP).
  - 632/22 (= 621-643nm) 48mW (e.g. FRFP).
- Structured illumination microscopy (SIM, laser illumination):
  - 405nm 100mW
  - 488nm 150mW
  - 568nm 480mW
  - 642nm 500mW
- Total internal reflection fluorescence (TIRF, laser illumination, as above) can be used for Deltavision Localization Microscopy (DLM, similar to dSTORM), not covered in these instructions

Objectives:
Clean the objective before, during (for oil change) and after microscopy session. Use lens paper to remove excess oil, then cotton swab dipped in chloroform (roll once over lens), 2 to 3 fresh swabs per cleaning.

For all applications except TIRF: Olympus 100x/1.4 oil, I.D. 10007

For TIRF only: Olympus 100x/1.49 oil TIRF with correction, I.D. 10111

Polychroic changer (drawers):

1. BGR: blue, green, red and far-red emission paths to cameras 1 and 2
2. CYR: CFP, YFP and red emission paths to cameras 1 and 2 (note that we don't have the excitation lasers for CFP and YFP)

Emission filters on "East" camera 1:

1. polarizer (DIC)
2. BP 541/22
3. BP 609/37
4. BP 683/40

On "West" camera 2:

1. BP 478/35
2. polarizer (DIC)
3. BP 528/48
4. BP 436/31

Startup if all hardware components are on (see separate document for cold start):

1. If not already on, turn on the two computers under the desk:
    - Image Processing **Linux Workstation** (also called omxSI)
      User: <worx>
      Password: <OMX023SI>
    - Master Controller **Windows Workstation** (also called omxMaster)
      use personal account user name and password

    If the computers have been off, wait about five minutes for all components to communicate.
2. On omxSI (Linux), click the Start softWoRx icon to open the **softWoRx software**.
3. On omxMaster (Windows), click the OMX icon to open the **OMX software**.
4. Restart the hardware (not obligatory, but recommended):
    - In the OMX V4 software, click **Instrument|Status.**
    - In the dialog box, click **Restart Hardware** to initialize the system hardware.
    - Once the hardware is fully initialized (about two minutes), the Status section of the main program window will change from displaying *HW: Restarting* to *HW: Running* and the system will be ready to acquire images.
5. Turn on the **lasers** required for your experiment on the Laser Control Module in the electronics rack. Turn on the key switch for the **interlock** that enables the laser safety shutter.

System Shutdown between experiments/users

1. Turn off the key switch for the **interlock** to disable the laser safety shutter.
2. Turn off any **lasers** that have been turned on.
3. Close the **OMX software** on the Windows workstation.
4. Log out of **omxMaster** (Windows).
5. Close softWoRx software, log out of **omxSI** (Linux), or stay logged on.

Moving data off the acquisition computer

Data is always acquired to the ‘data1’ drive on the Linux computer, which is mapped as Z: on the Windows computer. Data should be copied (and then moved off this computer) as soon as possible, using one of three methods:

1. From the **Linux computer** via **intranet** (gFTP) to the **CIAN server** (10.1.0.3).
2. From the **PC** via **internet** (WinSCP) to the **CIAN server** (132.206.213.90)
3. ONLY from the **PC** to a **USB drive**
   Important: **Do NOT connect a USB drive to the Linux computer!**

Please remove old data as soon as it is safely stored somewhere else.

Immersion oil (starting suggestions):

- Put the immersion oil on the cover slip, not the objective.
- Start with refractive index of **1.512** when **green** emission is critical, **1.514 or 1.516** when **red** emission is critical. Adjust for higher wavelength when attempting super-resolution in several colors.
- Readjust if necessary; change oil RI for:
    - imaging deeper in sample, i.e. away from cover slip (raise RI),
    - change in temperature (raise RI for raise in temperature)
    - spherical aberrations (asymmetrical Z signal above/below focal plane in +/- 2µm), raise RI if ringing is below focal plane, lower RI if ringing is above focal plane

Positioning on a region of interest:

1. The region of interest on the slide has to be in the center of the whole slide.
2. Move around in X and Y with "dX" and "dY". A value of 10µm is for small movements, 40 for an entire field of view, above 500 for larger steps, e.g. going to center of slide before touchdown. (XY movement is also possible by dragging the little green square in the stage display.)
3. In the "Nano Positioning" section, find a "Z touchdown" which is the closest to your sample. Position 24400 is where the stage is all the way up (e.g. to change the slide, or at shut down).
4. Focus up and down with "dZ". A value of 1 is a fine focus, a value of 1000 is a very coarse focus; units are micrometers. (Z movement also possible by dragging the yellow line in the stage display.)

Recommended starting settings for image acquisition:

- Select sequential mode, 512 x 512 format.
- Click on the channel letter to activate it. Select the appropriate excitation laser.
- EM CCD: 5 or 10MHz, Gain 170, 5 to 100 ms exposure (e.g. 50 ms).
- Laser intensity (%T) 10%. A higher laser intensity may help finding out of focus signal, but be careful not to saturate/damage the cameras.
- Look for maximum pixel intensity values of ideally about 40 000 gray levels, or at least 10 to 20 000, if bleaching is a concern).
- "Spiral mosaic" will help finding the right field of view.
- Experiment type: SI, 0.125um spacing, enter stack thickness or "get thickness" from top/bottom settings, move stage to middle of stack before starting experiment

Other things to remember for acquisition:

- Pixel size of raw data is 80nm/pixel.
- Acquired data is saved directly into *data1* on the Linux computer, mapped as the *Z*: drive on the Windows computer.

Generating the PSF/OTF (typically done by CIAN personnel)

1. Prepare bead slides of sub-resolution beads (100 nm “Fluorospheres”, Molecular Probes) in red and green, dried on cover slip, mounted in glycerol.
2. Find single bead that is located directly on the cover slip.
3. As experiment type, use “SI PSF”, with PSF angle of 1 (or collect one for each angle and pick the best).
4. 8 µm thick, 0.125 µm Z spacing, 256 x 256 pixels.
5. Adjust exposure time and laser intensity to get maximum pixel intensity values of 10 to 40 000 gray levels.
6. Generate OTF:
   Minimum of 9 slices, 0.218 µm line spacing.
7. Clean OTF according to API instructions.
8. Perform test reconstruction on field of (multicolor) beads.
9. Look for warning messages and inspect the reconstructed image (FWHM).

SI Reconstruction (use on raw SI data only!)

- In SoftwoRx, open Process/OMX SI Reconstruction, select input file
- Parameters:
  - **OTF**: for multiple colors, channel-specific recommended; select drawer.
  - **Wiener filter** constant: default value 0.001 useful for most applications, can be adjusted up (to 0.002 or 0.003) to smooth signal at the expense of resolution; may be set for each channel.
  - **Background intensity offset**: make it lower than the lowest intensity measured in your raw image stack (can be read from the contrast/brightness window in SoftwoRx, should not be negative); if channels are very different, use channel-specific background values
  - **k0 angles**: use channel-specific values when working with more than one color; select drawer
- More options:
  Discard negative intensity values (keep it selected).
  Keep “Do drift correction” unchecked.
- Always check SI reconstruction log for warning messages (e.g “Best fit for k0 is 182.91111 pixels from expected values”).
- Pixel size of reconstructed data is 40 nm/pixel (~20 µm x 20 µm for each frame).
- Resulting image: …**_SIR.dv**

Image registration to correct for chromatic aberration and camera alignment

Note: do after SI reconstruction!

- SoftWorx main menu, Process/OMX Image Registration
- "Shift and rotate" method
- Select drawer
- Do It
- Output file: …**_ALX.dv**

Task builder to queue tasks for processing one or more files

- SoftWorx main menu, Process/Task Builder
- Select tasks, e.g. SI Reconstruction and Image Registration
- Set parameters (note that some are not available in this interface, such as channel-specific Wiener filter constants)
- Load file(s) from data folder, not image windows
- Run

Conversion of SI raw data to Widefield-like data by averaging phases

- SoftWorx main menu, Process/Generate Widefield Image…
- Define input file, accept default values (average 5 phases of direction 1)
- Output file: … **_WF.dv**

- Alternative method:
    - SorfWorx main menu, View/Quick projection, input: raw SI data window.
    - Method: select "Average".
    - Number of Sections to Average: unselect "All", put in 5 (for the 5 phases within the angle).
    - Details/Output Options/Z start: put in the total number of Z slices in the raw data divided by 3 (for first one of the three angles of the grid rotation). Close the dialog box.
    - Click "Do It".
    - Save resulting image (…**_PRJ.dv**)
    - To re-adjust Z spacing from 0.625 to 0.125 µm: Edit/Header, input: newly saved file, not image window
- Deconvolve (optional); resulting image: ...**_D3D.dv**

Miscellaneous notes:

- In conventional non-SI mode, use Z-spacing of 0.2um.