**Quick starter check-list for B24 staff on day of data collection**

At B24 synoptic (B24 staff only):

Open ‘Front End Status’--> ensure all absorbers are out --> take exit slits to 60x60microns Y and X gaps--> PGM should have Energy 500eV; Cff 1.6 and PGM Control to show Grating Translation 800l/mm; Mirror Translation Nickel

At the TXM (B24 staff only):

Make sure both VBG and VM2 are open at the TXM

Check that GM1 and GM2 are at high vacuum

Check ‘Cold Sample Chuck’ temperature is below 114K

At the TXM PC: For start data backup (B24 staff only)

Start Cygwin64 Terminal (located on the Desktop)

$ cd /cygdrive/d/data/<year>/<user\_visit>/

$ ~/transfer.sh <user\_visit>

Minimise application and do not close during data collection.

When collecting data, it must be saved to D:\data\<year>\<user\_visit>\ (or folders within this directory) or it will **not** be processed.

To stop the script, press `ctrl+c` with the Cygwin64 window selected.

**Data collection using XRM Data Explorer**

Start XRM Data Explorer (located on the Desktop) --> allow it to make changes

Double click on the XRM Explorer Python module (top left key panel) and minimise the panel (do not close)

Start Cyberlink YouCam (located on the Desktop)

Start Sample Loader (located on the Desktop) --> minimise extra black panel --> Scan samples --> click yes when asked if you are sure --> wait until Procedure Status reads ‘Idle’

*Note: all sample positions should register a ‘Sample+Cover’ state. If there is a position designated as empty, then that sample is already loaded on the sample stage.*

Unloading a sample

If there is a sample loaded press unload --> inspect the process through Cyberlink YouCam --> wait until Procedure Status reads ‘Idle’ -- > Close Cyberlink YouCam

Loading a sample

Click ‘Load sample’ and chose the appropriate carousel position --> inspect the process through Cyberlink YouCam --> wait until Procedure Status reads ‘Idle’ -- > Close Cyberlink YouCam --> minimise the loader panel

**VLM imaging**

**Move optics to safe positions**

* Open the motion control window (*joystick button*)  
    
  **CRITICAL:** On image control, do not confuse the step tab with the requested value tab or the current position tab. If you are not sure where these are, please ask!
  + *Sample --> Sample Theta = Set to -60 deg in the GO tab and press GO.*

**VLM imaging - Brightfield**

* Select camera (top panel camera tab = *magnifying lens button*)
  + *Choose VLM 20x*
* Centre grid
  + *Motion Control (top panel cogs tab) > Sample > Go to Sample X=100 > Go to Sample Y=*300
* Start continuous acquisition (Acquisition Settings = *gears button*)
  + *Mode continuous*, 0.2 s exposure
  + Source settings --> tick transmission --> start
* Use *Motion Control > Sample > Sample Z* to get the grid into focus
  + *Start with 100 um steps, then gradually reduce to 10 um steps as you approach focus*
  + *Just get to the point where you can see the grid letters clearly (or holes in grids),*
  + *abort acquisition*
  + *close image (do not save)*
* Collect a mosaic of the whole grid (*gears button*)
  + *Mode mosaic*
  + *camera settings 0.2 sec*
  + *source settings transmission ticked*
  + *Sample settings pixel size 0.37*
  + *Mosaic horizontal step should read 323.75 Vertical step 323.75*
  + *Mosaic Rows 7 Columns 7*
  + *Mosaic axes sample X and sample Y*
  + *start acquisition*
  + *Click Yes to the current rotary stage angle*
  + *Save in Local Disk D:/<user\_directory>/<sample\_specification>VLM\_grid\_mosaic*
  + *On the image histogram panel (bottom left) select ‘Full range’ and chose histogram region to suit (left click and drag in the histogram view)*
* Annotate with AOIs
  + *Frame AOIs with box tool from the side panel attached to the current image*
  + *Use line tool to number if desired*
  + *Save mosaic as VLM\_grid\_mosaic\_annotated*
* Focus on AOI
  + *Place pointer in the centre of the area*

**CRITICAL: Make sure that the slider at the bottom of the mosaic is at frame 1**

* + *Right click with mouse*
  + *Choose Go to Position option from the dropdown menu*
  + *Accept new position by pressing Go*
  + *Start continuous acquisition*
  + *fine focus using sample Z (start with small steps of 20)*
  + *Once in focus, abort acquisition.*
  + *Save image as VLM\_BF\_<GridLocation = LetterNumberLetter>*

**VLM imaging - Epifluorescence**

**At the TXM (Note: These steps can only be done in person at the beamline)**

* Plug in the LED light source (near the microscope tank)
* Set the LED channel and filter wheel to the appropriate colour
  + For green fluorescence use LED channel 3, Filter 4
  + For red fluorescence use LED channel 2, Filter 1
* Make sure all view ports are covered - I.e. turn off the bright field lamp and cover the aperture with foil.

**At the PC (back to Data Explorer)**

* Start continuous acquisition (Acquisition Settings = *gears button*)
  + *Mode continuous*
  + Camera Settings 20s (5 -> 60s, depending on sample)
  + Source settings --> untick transmission
  + Start
  + Abort when happy with exposure (binning will increase your signal if weak)
* Collect a focal series to find focus
  + *acquisition mode focal series*
  + camera settings
    - Exposure 5s
    - binning 2
  + source settings
    - none chosen
  + focal series
    - total projections 10
    - images per projection 1
    - focal axis sample Z
    - range 10
  + Start and save as *FS\_<number>.txrm*

Note: to see the series in real time tick the box next to the slider at the bottom of the image

* + *when done move the slider to the best focus slice*
  + *position pointer in centre of view --> right click --> chose the go to position option and confirm*
  + *acquisition mode single, 10s exposure (or best estimate), binning 1*
  + *save image VLM\_FL\_<colour>\_<GridLocation = LetterNumberLetter>*

Note: The arrow at the top of the side panel will expand the image menu if desired

* Repeat for other channels If needed (no further focusing required)

**At the TXM (To be done in person at the beamline)**

* Unplug the LED light source (left most cable at the back of the controller box)

**Xray imaging**

**Move optics to safe positions**

* Open the motion control window (*joystick button*)  
    
  **CRITICAL:** On image control, do not confuse the step tab with the requested value tab or the current position tab. If you are not sure where these are, please ask!  
  + *Sample --> Sample Theta = set to 0 (zero) degrees in the Go tab and then press Go*

**At the TXM (To be done in person at the beamline)**

* Top up the cold trap with LN2 and wait for 5-10 mins.

**X-ray imaging**

* Microscope tab (top left of the main panel) --> Camera temperature --> Pixis --> change set temperature –65C --> apply
* Select camera (top panel camera tab =magnifying lens button)
  + *Choose Pixis 1x*

**Reference Acquisition**

* Select location
  + open VLM grid mosaic
  + Select area on the VLM grid that has no membrane or cells *(make sure image slider is at 1)*
  + *position pointer in centre of area --> right click --> chose the go to position option --> change theta to 0 degrees --> and Go*
  + *Start condenser agitator by clicking on the green line button at the top view panel (if it shows as a sin function it already is on)*
  + *acquisition mode continuous, 0.5s exposure, sample settings pixel tick, pixel size 0.01*
  + *(if a clean Lissajous pattern is obvious) motion control --> condenser Z --> step size 200 --> move one step minus to defocus the beam. Check that the lines of the Lissajous pattern are fuzzy rather than sharp.*
  + *Motion control --> condenser X, step 0.2-1, move plus or minus to centre pattern*
  + *Motion control --> condenser Y, step 0.2-1, move plus or minus to centre pattern*
* Acquire reference
  + *acquisition mode average, 0.5s exposure, sample settings pixel 0.01, total projections 10, Images per. projection 1, collect reference none, Average on the fly not selected*
  + *Use name Ref\_<date as yyyymmdd> \_<time as hhmm>*
  + *Start acquisition (note the image is not updating unless the bottom panel box on the image series is ticked)*
  + *Process image (top left of main view), average series of Images..., choose input, tick despeckle, start*
  + *Save averaged*

**Xray mosaic of ROIs**

* Find ROI
  + open focused VLM of ROI (brightfield or fluorescence)
  + *position pointer close to features of interest --> right click --> choose the go to position option --> change theta to 0 degrees --> and confirm*

*Note: To choose a starting area ensure you are not on the thickest area of a cell and not near a grid bar; stay close to the biological material*

* Start acquisition (*gears button*)
  + *Mode continuous*, 0*.5s exposure*
* Move *Motion Controller --> Sample --> Sample Z* to get sample into approximate focus (1-5u steps )
* Focus on support film or cell features
* Abort when in focus
* Start acquisition (*gears button*)
  + *Mode mosaic*, Camera setting 0*.5s, binning 1, Sample settings 0.01,*
  + *Mode mosaic, mosaic--> images per projections 1-->horizontal step*
  + *vertical step 9.46 --> Row 7, columns 7, horizontal axis sample X, vertical axis sample Y*
  + *Save as xray\_mosaic\_<GridLocation = LetterNumberLetter>*
* Reposition and repeat if a shift in view is required
* Reference mosaic
  + *Go to image and select the arrow button. (top left) to expand the metadata*
  + *Choose the reference tab --> single reference browse, chose the correct file, apply single reference*

**Tilt series acquisition**

* Centre on ROI
  + open X-ray mosaic of the area with marked selected FOV
  + *Place pointer on desired centre --> right click --> chose the go to position option --> change theta to 0 degrees --> and confirm*
* Find optimal exposure for projections
  + *Acquisition settings: mode continuous, camera setting 0.5s, start*
  + *Check pixel counts on your image (10-30k on average is good); change exposure in increments of 0.5sec to increase count if needed*
  + *Acquisition settings: Abort and delete image*
* Move to tilt axis
  + *Acquisition settings: mode continuous, camera setting 0.5s, start*
  + *Motion Control --> Sample --> Sample Z* to get sample into approximate focus (in 0.5-1u steps) if needed
  + *Motion control, go to sample theta= –30*
  + *Use line tool to mark a distinct and tractable fine feature*
  + *Motion control, go to sample theta= +30*
  + *Use line tool to mark the same point*
  + *Motion Control --> Sample --> Sample Z; use steps of 0.2-1u to move the same feature perfectly between the two lines*
  + *Motion control --> Sample theta = 0*
  + *Acquisition settings: Abort and delete image*
* Focus the objective
  + *Acquisition settings: mode focal series = camera setting 0.5s, binning 1; Focal series = Images per projection 1, wait between images 0.2, Focal axis Zoneplate Z, Range 10, Start*
  + *Save as FS\_<IncreasingNumber>*
  + *Choose best focal slice and remain on that slice*
  + *Go to the forward arrow button ‘>’ on the right of the tilt series and select Positions --> Note down the Zoneplate Z Actual position*
  + *Go to the motion controller --> Zoneplate --> Change Zoneplate Z to the recorded value and click GO*
  + *Check that the sample has moved to the desired Z coordinates by running a continuous acquisition.*
* Test maximum rotation
  + *Acquisition settings: mode continuous, camera setting 0.5s, start*
  + *Motion Control --> Sample --> Sample theta step =5, Sample theta =50*
  + Step sample theta down to –60 and up to +60. Record extremes with clear visibility (grid bars or sample density can obstruct light at high tilts).
  + *Note pixel counts at high tilts and consider increasing exposure if counts are low (<3,000)*
  + *Acquisition settings: Abort and delete image*
* Decide on data collection strategy (depends on sample stability and user project needs and desperation)

*Conservative: 1deg steps, 0.2 delay, (ie -55 to +55: 111 images)*

*Standard: 0.5deg steps, 0.2 delay (ie -60 to +60: 241 images)*

*Exhaustive: 0.2 deg steps, 0.2 delay (ie –55 to +55: 551 images or –60 to +60: 601 images)*

**CRITICAL: Be extremely cautious when changing Sample Theta. Never exceed -60 to +60 degrees range (-60 =< theta <= +60).**

* Collect tilt series
  + *Acquisition settings:*
  + *Acquisition Modes: Tomography*
  + *Camera Settings: 0.5-5s, binning 1*
  + *Sample Settings: tick pixel size = 0.01*
  + *Tomography: images per projection 1, wait between images 0.2, total projections <YourTotalNumberOfImages>, Angle Start= –60, Angle End= +60, Return to zero degrees when finished = ticked, collect reference none, variable exposure none, start*
  + *Save as <TiltSeriesNumber>\_Tomo\_<GridBox>\_<GridStoragePosition>\_<GridArea>\_<NumberOfFOV> (i.e. 01\_Tomo\_K1\_G1\_D4A\_ROI1)*
  + *When finished, click on arrow top-right at Image side panel --> Reference: load single reference, Shifts: uncheck Dynamic runout*
  + *Save Tilt Series*

**Using XRM controller**

**Sample loading**

Go to Microscope--> Cryo sample Control --> Scan samples --> wait until the procedure status reads ‘Idle’

* If there is a sample loaded at imaging stage, one of the positions on the shuttle will read ‘Empty’

--> Unload Sample --> wait until the procedure status reads ‘Idle’

* If there is no sample loaded at imaging stage, all positions on the shuttle will show as ‘Sample+Cover’--> close Sample control
* --> Load sample --> chose the position you want to load --> wait until the procedure status reads ‘Idle’ -> close Sample control

**Note:** Inspect all sample moves through Cyberlink YouCam --> wait until Procedure Status reads ‘Idle’ -- > Close Cyberlink YouCam

**Imaging with visible/white light & grid mapping (20x; sample theta=60deg)**

* Select the VLM camera (Microscope > Camera > VLM)
* Set *Motion Controller > Sample > Sample X 100 and Sample Y*  300µm to centre the grid
* Turn on Transmission illumination (*flashlight button > transmission illumination*)

*To avoid optics collision*

* Set Motion controller -- > go to zoneplate --> set Zoneplate Z step 5000 --> Move Zoneplate z by +5000 (to appx –9078)
* Set *Motion Controller > Sample > Sample Theta* to -60 deg
* Set Motion controller -- > go to VLM --> set VLM X step 500 --> Move VLM X by –500 (to appx -3692)

*cont.*

* Start continuous acquisition (*gears button*)
  + *Mode continuous*, 0.2/0.5 s exposure, binning 1
* Open the motion control window (*joystick button*) is open
* Use *Motion Controller > Sample > Sample Z* to get the grid into focus
  + Start with 100 um steps, then gradually reduce to 10 um steps as you approach focus
  + Just get to the point where you can see the grid letters clearly (or white holes in grids), stop acquisition and close image.
* Collect a mosaic of the whole grid (*gears button*)
  + *Mode mosaic*, keep default settings --> start acquisition
* Open image control window (*hammer/spanner button*) and histogram window (*histogram button*)
* Reset contrast [Histogram > Reset button] -- > select area of contrast manually
* Use *Image Control > Annotation* to mark the grid squares you will want to image
* Make a new directory for this grid, name /user-visit/raw/<Gridbox\_position>
* Save image as Grid\_mosaic\_<Gridbox\_position>

**VLM focusing in AOI**

* move to the desired region of the sample
  + Use *Image Control --> Annotation*
  + Press the button that looks like an excel spreadsheet then click on AOI on the VLM image
* Start continuous acquisition (*gears button*)
  + *Mode continuous*, 0.2/0.5 s exposure, binning 1
* Use *Motion Controller > Sample > Sample Z* to get the grid roughly into focus
  + Start with 20 um steps and move to smaller sizes to focus
  + **If you can't see the grid, then STOP and get the local contact - only move sample Z if you can see the grid!**
* Save as VLM\_BF\_<Gridbox\_position>\_<AOI>
* Close last image
* Repeat for all AOIs
* Move to the desired focused AOI image
  + Use *Image Control --> Annotation*
  + Press the button that looks like an excel spreadsheet then click in the middle of the view

*To return optics to X-ray imaging*

* Use *Motion Controller > Sample > Set sample theta 0 (zero)*
* Set Motion controller -- > go to VLM --> set VLM X step 500 --> Move VLM X by +500 (to appx -3192)
* Set Motion controller -- > go to zoneplate --> set Zoneplate Z step 5000 --> Move Zoneplate z by -5000 (to appx –14078)

Xray Data collection

* Swap to the Pixis camera (Microscope > Camera > Pixis)
* Make sure the motion control window (*joystick button*), image control window (*hammer/spanner button*) and histogram window (*histogram button*) are open

**2bi) Collecting Reference/Average (this should be recollected every 2 hours or after a grid has been changed)**

* Find some area with hole on the grid for recording the background image
* change to -60 theta (because it’s VLM)
* Press the button that looks like an excel spreadsheet and click on the closest gap/hole on VLM picture to go there
* change to 0 theta, record and save reference/background image (Gears button-averaging-1s- 20 images)
* Go to process-average-despeckle and average then save the despeckled image of reference/average
* Apply despeckled background correction by clicking on one zoomed Pixis view (of the area of interest) then click on the Image Control with right mouse and go to reference: select the despeckled reference/average image

**OR**

* If you are still in the same grid and have an old reference, open it and click on it by pressing the button that looks like excel spreadsheet
* Collect average of 20 images again and despeckle the average

**NB: If an area in the grid is out of focus in the VLM, don’t do x-ray mosaic right away, go back to VLM and focus before focusing with x-ray. This helps avoid radiation damage**

* Start continuous acquisition (*gears button*)
  + *Mode continuous*, 0.5 s exposure (or multiples of 0.5), binning 1
* Move *Motion Controller --> Sample --> Sample Z* to get sample into approximate focus (2 uM steps)
* Stop acquisition (*Stop button*)
* Close image window and don't save image

* Collect an X-ray mosaic of the area (*gears button*)
  + *Acquisition Mode mosaic*, keep default setting ( 7 x 7 grid, 0.5 s, binning 1 to get the whole grid)
* Right clicking on *Image Control* then click *Reference, Single Reference, Browse* and select most-recent reference file (go to section 2bi below for how to collect reference)
* Click *Auto* in the histogram window to reset the brightness/contrast
* Select the box in *Image control* and draw a box on the mosaic that's just smaller than one camera field (square)
* Move box onto areas of interest after clicking the arrowhead icon (copy and paste for multiple areas of interest)
* Annotate using the line tool the order in which you plan to collect the fields
* Save image as Xray\_mosaic\_<Gridbox\_position>
* Click reset in histogram to see referenced mosaic

**3) TILT SERIES/TOMOGRAM CAPTURE**

**3a) Focusing**

* Open the X-ray mosaic image
* Use *Image Control > Annotation* to move to the desired region of the sample
  + Press the button that looks like an excel spreadsheet then click on desired spot of the mosaic

* Start continuous acquisition (*gears button*)
  + *Mode continuous*, 0.5 s exposure (or multiples of 0.5), binning 1
* Set *Motion Controller > Sample > Sample Theta* to -30 deg
* Use *Image Control > Annotation* to draw a vertical line on a feature in centre of the field like a lipid droplet (use *Shift button* to make line straight)
* Set *Motion Controller > Sample > Sample Theta* to 30 deg
* Use *Image Control* to draw a vertical line
* Move feature to the centre of the two lines by changing *Motion Controller > Sample > Sample Z,* using small steps 0.1 -1 μm
  + If θ > 0, positive Z moves go left, negative go right
  + If θ < 0, positive Z moves go right, negative go left
  + If necessary, draw a horizontal line between vertical lines to help find the mid-point
* Stop acquisition (*Stop button*)
* Close image and don't save image
* Set *Motion Controller > Sample > Sample Theta* to 0 deg

**3b) Focal series**

* Collect a focal series to find focus (*gears button*)
  + *Mode focal series*, range 10, No of images 10, 0.5 s exposure, binning 1, **Zoneplate\_Z**
  + Save image with a numerical identifier (e.g. "FS\_2")
* Manually go through image stack to find image with best focus (look at features in cell)
* Right click on *Image Control* then click *Focus series, Plot focus function*
* Click on data point for image you thought was best (don't trust the computer)
* Close image window

**3c) Experimental limits**

**3ci) Finding rotation limits**

* Start continuous acquisition to find rotation limits (*gears button*)
  + *Mode continuous*, 0.5 s exposure (or multiples of 0.5), binning 1
* Set *Motion Controller > Sample > Sample Theta* to -60 deg and then step forward/backwards in 5 deg steps to find extreme limit for rotation
  + Make sure you have more than ~1500-2000 counts in the area of interest and < 65536 in any part of image
  + **NEVER go beyond -70 deg**
* Set *Motion Controller > Sample > Sample Theta* to -60 deg and do the same (**NEVER go beyond 70 deg**)
* Stop acquisition (*Stop button*)
* Close image and don't save image
* **NB: If the sample is prone to radiation damage, find rotation limit by single acquisition after you are at desired sample theta**

**3cii) Finding exposure time limit**

* **This needs to be done once per sample/grid. This also needs to be done if attenuator filter is brought in.**
* **Take single images at 0.5s, 1s, 1.5s and 2s exposure and compare resulting images side by side.**
* **Settle for an exposure where you can see biological features clearly with enough count as well. Do not choose exposure whose counts exceeds 60,000**

**3d) Tilt series/Tomogram collection**

* Make sure the nitrogen in the dewar tank is 70% full, then return it to auto/close mode
* Start tomogram acquisition (*gears button*)
  + *Mode tomography*, 0.5 s exposure (or multiples of 0.5), binning 1
  + Start angle and End angle as determined above (finding rotation limits)
  + No of images = (Angular range /no of steps per deg) + 1
    - Possible steps 3, 2, 1, 0.5, 0.2 (for fine phi slicing) step(s) per degree
* Save acquisition with following filename:
  + <index>\_Tomo\_<gridbox\_position>\_<grid\_coordinate>\_<field number>
* Put Liquid Nitrogen to auto mode
* Right click on *Image Control* then click *Shifts* and Untick *Metrology shifts*
* Right clicking on *Image Control* then click *Reference*
  + *Single Reference, Browse* and select most-recent reference file
* Close the image window
  + Image should have automatically saved, but if not then save when prompted

**Quick end of beamtime shift checklist**

* Change front end (FE) status to partially close
* Warm up Pixis camera to 23ºC
* Make sure LN2 is set to auto

**TXM calibration - first day of visit**

*Note: These steps assume that the TXM has been recently fully calibrated; A Wtip is handy to leave on the sample carousel in case further axes alignment is needed unexpectedly; should something go wrong a Wtip will help with stage rotation and camera alignment; accurate pixel calibration is only possible with a test pattern.*

Maximise XRM Data Explorer

Go to Microscope --> Microscope Mode --> Direct beam

Go to Microscope --> Microscope Mode --> Retiga Phoshor Screen IN

Set detector (magnifying glass symbol) to ‘Retiga Phosphor Lens’

Open Acquisition Setting (cogs symbol) --> set Acquisition Mode Continuous; Exposure Time 0.05 --> Start

On the Histogram panel (bottom left) select Full range

On the Image View --> Use the magnifying lens (plus button at bottom left) to zoom on the beam

**Beamline focal position optimisation**

At the B24 Synoptic

Open FEM --> at the Pitch tab enter step size of 0.02 --> step Pitch plus/minus until the beam is at maximum spread on the XRM Image View--> record the new Pitch value --> save the TXM image for future reference

**TXM Focus optimisation with Wtip**

Go to sample loader panel --> load sample --> Wtip position --> inspect the process through Cyberlink YouCam --> wait until Procedure Status reads ‘Idle’ -- > Close Cyberlink YouCam

Go to Microscope --> Microscope Mode --> Optics Imaging 25nmZP RT 500eV --> inspect the process through Cyberlink YouCam -- > Close Cyberlink YouCam.

*To set a new Microscope Mode:*

*Go to Microscope Mode setup --> define positions --> add positions --> give it a name --> tick all necessary axes --> save (bottom right) --> add sequence --> name --> expand --> click plus sign --> choose the required sub-sequences --> hit ok --> choose sequence of events --> save --> add a mode --> name it --> OK --> expand --> leave with Retiga (for now can only use 1st X-ray camera from main dropdown menu) --> pick the correct elements --> save --> move to the top according to complexity --> save*

*To update*

*Bring all motors to new positions --> Go to Mode setup --> expand the one to be updated and click update positions --> save*

*To configure user limits*

*Microscope --> motion --> configure user limits --> user soft limits --> enable by ticking the box and apply*

go to Microscope --> Microscope Mode --> Retiga Phoshor Screen IN

Set detector (magnifying glass symbol) to ‘Retiga Phosphor Lens’

Acquisition Settings --> Acquisition mode Continuous --> Exposure Time 0.05

Open the direct beam view and define size with a rectangle shape --> copy the shape and paste in active view -->

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go to Microscope --> Microscope Mode --> Retiga Phoshor Screen OUT

Set detector (magnifying glass symbol) to Pixis 1x

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go to Microscope --> Microscope Mode --> Retiga Phoshor Screen OUT

Set Detector (magnifying glass symbol) to Pixis 1x

Acquisition Settings --> Acquisition mode Continuous --> Exposure Time 0.05