Suggestions for Clearmap/TubeMap imaging

# Brain positioning

Typical positioning: medial side down, lateral up, anterior posterior axis perpendicular to light-sheet. Select light-sheet to come in from dorsal side. Imaging plane is sagittal.

# Acquisition settings for cFos staining

## UltraMicroscope II infinity-corrected optics or UltraMicroscope Blaze

### Cropping strategy

* 4X objective
* Cropping: 1000x1000 in center of field of view.
* Sheet NA = 0.026 (this number can be slightly different depending on the software version)
* Sheet Width = 100%
* Overlap between tiles = 10%
* Z step = 6µm steps
* Three sheets from one side
* Number of Tiles = approximately 7x5 matrix

This gives roughly 6um voxels.

40-50 minutes estimated acquisition time.

### Dynamic focus strategy

* 4X objective
* Cropping: none is fine, can be adjusted.
* Sheet NA = 0.026
* Dynamic focus on:
  + Use a few more steps than those recommended by the software (20-40% more)
  + At least 30ms of exposure, longer exposures avoids artefacts.
  + Dynamic focus range across entire field of view.
  + Dynamic horizontal focus processing: Blend. Note that this will only work properly if the sheetmotor calibration is set perfectly. Alternatively, contrast adaptive can be used.
* Sheet Width = 100%
* Overlap between tiles = 10%
* Z step = 6µm steps
* Three sheets from one side
* Number of Tiles = approximately 3x2 matrix

This gives roughly 6um voxels.

Acquisition time typically longer than with cropping strategy.

## UltraMicroscope II zoom body optics

### Cropping strategy

Modified from Renier et al 2016, tested at UNC.

* Zoom = 2.5x (5x total magnification with 2X objective). Note that this higher mag (compared to Renier et al 2016) is necessary to avoid distortions on edge of field of view visible with 2X zoom.
* Cropping: 1000x1000 in center of field of view.
* Sheet NA = 0.026 (this number can be slightly different depending on the software version0
* Sheet Width = 100%
* Overlap between tiles = 10%
* Z step = 6µm steps
* Number of Tiles = approximately 9x6 matrix

This gives roughly 6um voxels.

# Acquisition settings for vasculature

## UltraMicroscope II infinity-corrected optics

### Cropping strategy

* 4X objective
* 400 x 1300 crop
* 10% overlay
* 0.14-0.1 NA. Lower is more tolerant to slight misalignments in sheetmotor calibration.
* 2µm step size
* Single sheet illumination from one side
* Number of Tiles = approximately 12x6 matrix

This gives isotropic 2um pixels

### Dynamic focus strategy

* 4X objective
* Cropping: none is fine, can be adjusted if aberrations are seen on edges
* Sheet NA = 0.14-0.10. Lower is more tolerant to slight misalignments in sheetmotor calibration.
* Dynamic focus on:
  + Use a few more steps than those recommended by the software (20-40% more).
  + At least 30ms of exposure, longer exposures avoid artefacts.
  + Dynamic focus range across entire field of view.
  + Dynamic horizontal focus processing: Blend. Note that for highest NA, this will only work properly if the sheetmotor calibration is set perfectly. Alternatively, contrast adaptive can be used.
* Sheet Width = 100%
* Overlap between tiles = 10%
* Z step = 1.6-2µm steps
* Single sheet illumination from one side
* Number of Tiles = approximately 3x2 matrix

This gives isotropic 2um pixels

## UltraMicroscope Blaze

### Dynamic focus strategy

* 4X objective
* Cropping: none is fine, can be adjusted if aberrations are seen on edges
* Sheet NA = 0.14-0.10. Lower is more tolerant to slight misalignments in sheetmotor calibration.
* Dynamic focus on:
  + Use a few more steps than those recommended by the software (20-40% more).
  + At least 30ms of exposure, longer exposures avoids artefacts.
  + Dynamic focus range across entire field of view.
  + Dynamic horizontal focus processing: Blend. Note that for highest NA, this will only work properly if the sheetmotor calibration is set perfectly. Alternatively, contrast adaptive can be used.
* Sheet Width = 100%
* Overlap between tiles = 1%
* Z step = 1.6-2µm steps
* Single sheet illumination from one side
* Number of Tiles = approximately 3x2 matrix

This gives isotropic 2um pixels

# Acquisition settings for autofluorescence

Use lowest mag, low NA (0.03), three sheets from one side, Z step size 6 um, 488 nm channel.