Microscopy Imaging Session Log

Objective, Filter & Exposure Record

Session
Date: 2026-08-03
Session ID: IMC-0803-A
Operator: R. Souza
Reviewer: Dr. A. Okonkwo
Start: 09:12
End: 12:47

Sample
Sample ID: NX-SLD014
Specimen: HeLa, fixed
Mount: ProLong Glass
Slide rack: R3, slot 6
Replicates: n = 3
Stain set: DAPI / GFP / RFP

Instrument
System: Vertex Ti2-E
Camera: Apex sCMOS 4.2
Software: NIS-Elements 5.4
Bay: Imaging Bay 2
Env. control: Off (RT, fixed)
Vibration table: On

Purpose & Scope

This log records a single acquisition session on sample batch **NX-SLD014** (fixed HeLa monolayers, three biological replicates, immunolabelled for a nuclear marker, a cytoskeletal GFP reporter, and an RFP-tagged trafficking construct). The session combined a low-magnification survey pass across each coverslip to select fields of view, followed by high-magnification detail acquisition of six selected regions per replicate. All settings, file names, and calibration checks performed during the session are recorded below so that the resulting image set can be reprocessed or audited without access to the acquisition software.

Deviation noted: the metal-halide lamp required an additional 8-minute warm-up beyond the standard 15 minutes before excitation intensity stabilised on the GFP channel (see §5 for disposition). This delayed the first detail acquisition to 09:41.

1 Objective, Filter & Exposure Settings

Two acquisition passes were configured on the Vertex Ti2-E: a **survey pass** at 20× to locate healthy, well-spread fields, and a **detail pass** at 60× oil immersion for quantitative channel capture. Filter cube and camera settings for each pass are fixed for the duration of the session; any manual override during acquisition is logged separately in §2.

1.1 Survey Pass – 20× / 0.75 NA (dry)

Channel	Filter Cube	Ex / Em (nm)	Exposure (ms)	Gain (dB)	Binning
DAPI	DAPI-5060B	395 / 460	40	0	2×2
GFP	FITC-3540C	470 / 525	60	3	2×2
RFP	TxRed-4040C	555 / 605	80	3	2×2
Brightfield	–	Transmitted	12	0	2×2

1.2 Detail Pass – 60× / 1.40 NA (oil)

Channel	Filter Cube	Ex / Em (nm)	Exposure (ms)	Gain (dB)	Binning
DAPI	DAPI-5060B	395 / 460	120	6	1×1
GFP	FITC-3540C	470 / 525	200	9	1×1
RFP	TxRed-4040C	555 / 605	250	9	1×1
Cy5 (spare)	Cy5-6060C	640 / 690	–	–	not used
Brightfield	–	Transmitted	8	0	1×1

Illumination Notes

- Metal-halide source (Meridian X-Cite 200e), 60% neutral density filter engaged on all fluorescence channels.
- Detail-pass Z-stacks: 0.35 µm step, 15 planes, centred on best-focus brightfield slice.
- Autofocus: software contrast-based, GFP channel, run once per field before the stack.
- Cy5 cube left in the turret for a planned future far-red marker; not used this session.

2 Acquisition Log

The full session produced 312 raw TIFFs (3 replicates × 6 detail regions × 3 fluorescence channels, plus matched brightfield and survey frames). The table below is the operator's field log entered at the scope during acquisition – a representative subset covering the start, a mid-session recalibration, and the final region of replicate 2. The complete manifest is generated automatically by the acquisition software and stored alongside the raw data at `/data/imc/2026-08-03_NX-SLD014/manifest.csv`.

#	Filename	Ch.	Pass	Z	Notes
001	`20260803_NX-SLD014_R1_20x_DAPI_f01.tif`	DAPI	Survey	1	Field selected: even nuclear density
002	`20260803_NX-SLD014_R1_20x_GFP_f01.tif`	GFP	Survey	1	–
003	`20260803_NX-SLD014_R1_20x_RFP_f01.tif`	RFP	Survey	1	–
004	`20260803_NX-SLD014_R1_60x_DAPI_z01-15_t01.tif`	DAPI	Detail	15	First detail stack of session
005	`20260803_NX-SLD014_R1_60x_GFP_z01-15_t01.tif`	GFP	Detail	15	Delayed start, see §5
006	`20260803_NX-SLD014_R1_60x_RFP_z01-15_t01.tif`	RFP	Detail	15	–
007	`20260803_NX-SLD014_R1_60x_DAPI_z01-15_t02.tif`	DAPI	Detail	15	Region 2, replicate 1
⋮	⋮	⋮	⋮	⋮	*(regions 3–6 omitted, see manifest)*
118	`20260803_NX-SLD014_R2_60x_DAPI_z01-15_t01.tif`	DAPI	Detail	15	Stage micrometer re-check before R2
119	`20260803_NX-SLD014_R2_60x_GFP_z01-15_t01.tif`	GFP	Detail	15	Lamp intensity re-verified, 98% of AM reading
120	`20260803_NX-SLD014_R2_60x_RFP_z01-15_t01.tif`	RFP	Detail	15	–
⋮	⋮	⋮	⋮	⋮	*(regions 2–6 omitted, see manifest)*
312	`20260803_NX-SLD014_R2_60x_BF_z08_t06.tif`	BF	Detail	1	Session close, 12:47

⚠ Frame `005` exposure logged at 200 ms per protocol, but the lamp had not yet reached its stable output window (see deviation note, §5). Reprocessing may require a normalisation pass against the end-of-session lamp reading if quantitative GFP intensity is required for replicate 1, region 1.

3 Image File Naming Scheme

Every raw file from this facility follows a fixed eight-token pattern so that downstream scripts can parse metadata directly from the filename without reading embedded TIFF tags. The pattern is:

`YYYYMMDD_SAMPLEID_REP_OBJ_CHANNEL_zNN-NN_tNN.tif`

The worked example below is frame 004 from the acquisition log (§2), with each token colour-matched to its definition in the table beneath it.

Token	Example	Meaning
Date	`20260803`	Acquisition date, ISO 8601, no separators
Sample ID	`NX-SLD014`	LIMS slide/sample identifier
Replicate	`R1`	Biological replicate number on this slide
Objective	`60x`	Nominal magnification of the objective used
Channel	`DAPI`	Filter cube / channel name (matches §2)
Z-range	`z01-15`	First–last plane index of the Z-stack
Timepoint	`t01`	Field/timepoint index within the replicate
Extension	`.tif`	Uncompressed 16-bit TIFF, one channel per file

Survey-Pass Variant

Survey frames drop the Z-range and timepoint tokens in favour of a single field index, e.g. `20260803_NX-SLD014_R1_20x_GFP_f01.tif`. The `f`-prefixed field index is never reused across passes, so survey and detail filenames for the same physical location will not collide.

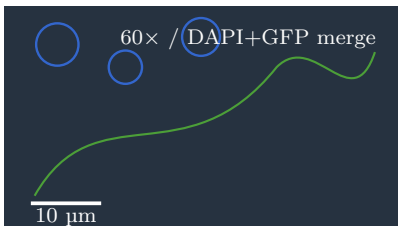
4 Scale-Bar Calibration

Pixel size was verified at the start of the session against a stage micrometer (10 µm divisions, NIST-traceable) rather than trusted from the objective's nominal magnification alone, following the general practice described by Waters (2009) and Pawley (2006). Each row below was captured as a dedicated calibration frame and stored under `/data/imc/calibration/2026-08-03/`.

Objective	Binning	Pixel Size (µm/px)	FOV (µm)	100 px Scale Bar
4×	1×1	1.625	2662 × 2246	162.5 µm
10×	1×1	0.650	1065 × 898	65.0 µm
20×	2×2	0.650	1065 × 898	65.0 µm
60×	1×1	0.108	177 × 150	10.8 µm

4.1 Worked Reference: 60× Detail Channel

The figure below is the calibration convention applied to every detail-pass figure exported from this session: a 10 µm bar corresponds to 92.6 px at 0.108 µm/px, rounded to 93 px in the exported overlay.



Calibration Disposition

- Stage micrometer check repeated before replicate 2 (frame 118) after the lamp warm-up deviation, per standing SOP to re-verify pixel size whenever a session exceeds 3 hours or the light path is disturbed.
- No drift detected: measured division width matched the AM calibration to within 0.4%, inside the ±1% tolerance in the facility SOP.
- Scale bars burned into exported TIFFs by the acquisition software are derived from this table, not recalculated per image – if the objective is swapped mid-session, this table must be re-run before further capture.

5 Session Notes, Deviations & Sign-Off

5.1 Deviations

1. **Lamp warm-up (09:12–09:41).** The metal-halide source did not reach stable GFP-channel output within the standard 15-minute warm-up. Frame `005` was captured at 09:22, before stabilisation; it was re-acquired at 09:44 as frame `005b` once intensity held within 2% over three consecutive readings. Both files were retained; the manifest flags `005` as *provisional*.

2. **Immersion oil bubble, replicate 1, region 4.** A small air bubble was visible in the oil layer during focus. The objective front lens was cleaned and re-oiled; the region was re-imaged and the original attempt discarded (not written to disk).

3. **Stage micrometer re-check, 11:58.** Performed ahead of schedule after the lamp deviation, per §4 disposition. No corrective action required.

5.2 Handover Notes

- Raw TIFFs copied to `/data/imc/2026-08-03_NX-SLD014/` and queued for nightly backup to the core facility archive.
- Analysis request logged separately in the core facility ticketing system as job `IMC-REQ-1142` (nuclear count + GFP intensity quantification, requested by Meridian Genomics Lab).
- Next scheduled use of Imaging Bay 2: 2026-08-04, 08:30 (Apex Labs booking).

Role	Name / Signature	Date
Operator	R. Souza	2026-08-03
Reviewer (facility)	Dr. A. Okonkwo	
Requesting PI	Dr. L. Fernandez, Meridian Genomics Lab	

Retain this log with the raw data set for the full retention period defined in the core facility data management policy. Cite Schneider et al. (2012) for downstream ImageJ/Fiji processing and Cole et al. (2011) if point-spread-function verification is required before publication.

References

Richard W. Cole, Tushare Jinadasa, and Claire M. Brown. Measuring and interpreting point spread functions to determine confocal microscope resolution and ensure quality control. *Nature Protocols*, 6(12):1929–1941, 2011.

James B. Pawley. *Handbook of Biological Confocal Microscopy*. Springer, New York, NY, 3 edition, 2006.

Caroline A. Schneider, Wayne S. Rasband, and Kevin W. Eliceiri. NIH image to ImageJ: 25 years of image analysis. *Nature Methods*, 9(7):671–675, 2012.

Jennifer C. Waters. Accuracy and precision in quantitative fluorescence microscopy. *Journal of Cell Biology*, 185(7):1135–1148, 2009.