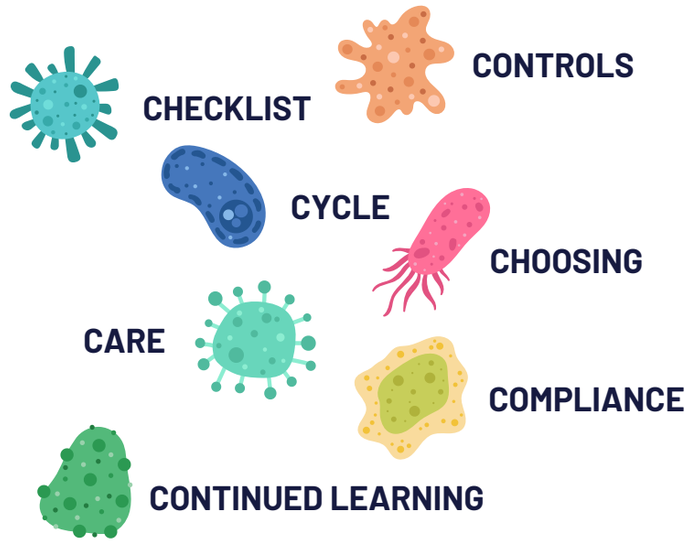
THE 7 C'S OF MICROSCOPY



1. IT'S ALL ABOUT CONTROL

Every part of your imaging experiment must be controlled.

Experimental Control

Experimental Controls and Technical Controls

- Autofluorescence
- Signal/Antibody specificity
- Single colour
- Functional probe controls...

and more

Acquisition Control

Controlled Acquisition

In order to compare image data sets, all imaging parameters must been kept constant. These include all light path components as well as detector settings.

Analysis Control

Analysis Workflow

In order to make meaningful conclusions, all analysis workflow steps must kept constant.
Your experimental and technical controls are necessary to validate your workflow.

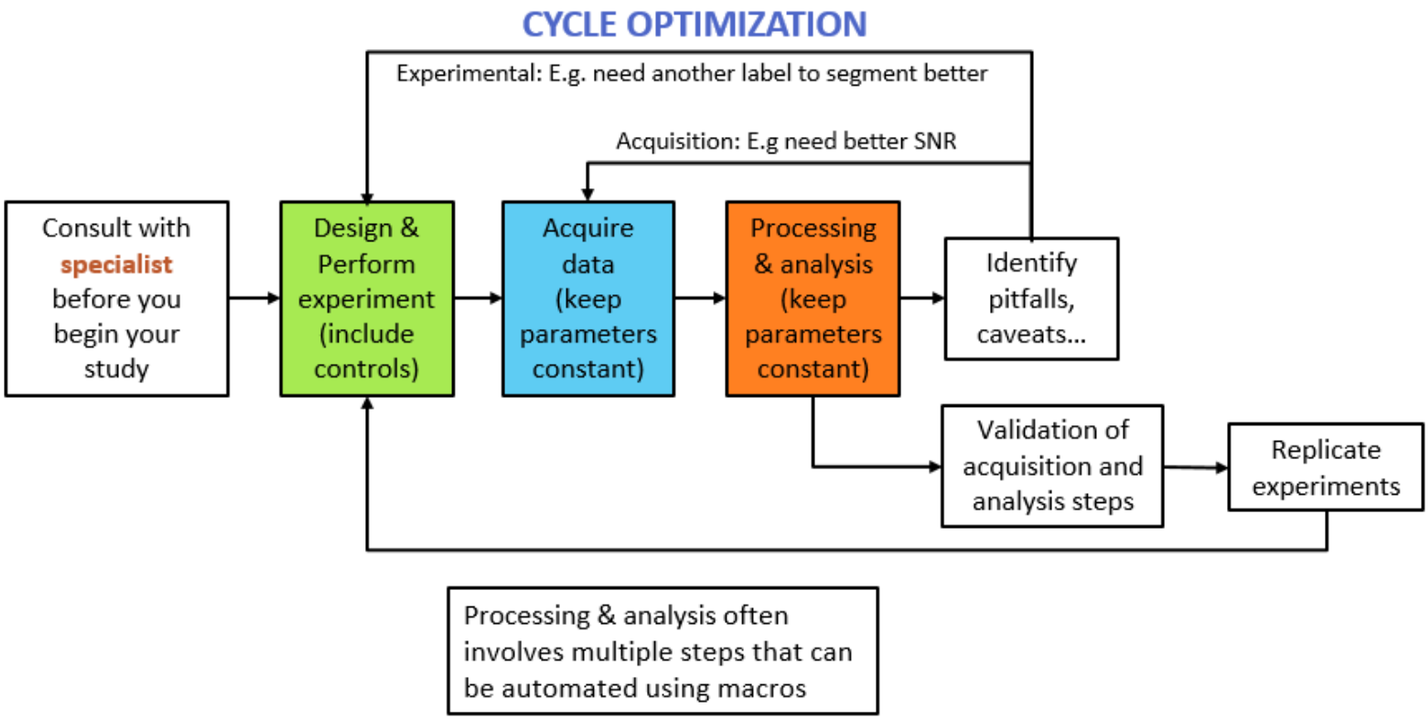
2. DATA ACQUISITION CHECKLIST

- Resolution and Nyquist Sampling
  - Are you using the correct NA lens to detect objects of interest?
  - Are you Nyquist sampling? If so,
    - For 2D - are your objects of interest at least 3 X 3 = 9 pixels (area)?
    - For 3D - are your objects of interest at least 3 X 3 X 3 = 27 voxels (volume)?
- Are your images within detector limits?
  - There should NOT be detector saturation
  - Data should be within the linear range of detector
- Have you examined your signal?
  - Signal to background ratio ideally 3: 1 or better
  - If your signal is noisy (shot noise) consider longer exposure times, averaging etc.
- Have you imaged your technical controls with the exact same parameters? This will tell you the following:
  - Is your probe/signal specific?
  - What is autofluorescence?
  - Do you have signal cross-talk/bleed through?
- Have you checked for the photostability of your probe?

RCF
RESEARCH CORE FACILITIES

3. IMAGING CYCLE

Imaging data can be highly quantitative if designed, acquired and analyzed correctly. Expect a few cycles to optimize this process.



4. CHOOSING COVERSLIPS AND MEDIA

COVERSLIPS

Most objective lenses are optimized to image through glass that is 0.17mm thick, so be sure to use high-quality #1.5 coverslips. This is especially important for: Confocal and Super Resolution Microscopy, TIRF, Deconvolution

<u>The exception to this</u> is lenses with correction collars (manual or automatic) that allow will adjust to the thickness of your sample.

Oil immersion only

Not adjustable

Multi immersion

Adjustable

CORRECT MEDIA RI MATCHING

Eliminating RI mismatch (i.e. between sample and lens) will result in significantly improved image quality and reduce optical artifacts.
Different objective lenses accommodate different immersion media (e.g. oil, water, glycerol) or no immersion media at all (air lenses).

Some objective lenses allow you to adjust to the RI of your sample (e.g. 10X glycerol lens for imaging of cleared tissue)

Fixed samples – use a mounting media that has the same RI as glass/immersion oil (do not image in PBS if doing high-resolution imaging)

Live samples (aqueous medium) – You may want to use a water immersion lens especially if your sample is thick. For example, samples embedded in agarose or Matrigel etc.

RI OF COMMON MEDIUMS

Substance	Refractive Index
Air	1.0003
Water	1.333
Glycerol (pure)	1.393
Oil (Type F)	1.518
Glass	1.517

5. MICROSCOPE CARE

Cleaning

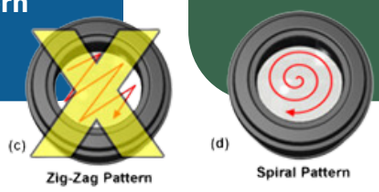
1. First pass – Use a dry Kim wipe to remove as much immersion media as possible
2. Second pass – Using a clean Kim wipe with 1-2 drops of the provided cleaning solution clean the objective with a circular pattern from center to rim
3. Third pass – repeat second pass
4. Fourth pass – Using a clean Kim wipe dry the objective with a circular pattern from center to rim

Collision-Avoidance

Collisions commonly occur when attempting to find focus and when the microscope is powered off in an awkward position.

Follow these steps before powering off equipment:

1. Center the stage
2. Move to lowest objective lens
3. Completely lower the lens



Leak Test

If performing live imaging you must check for leaks.

Leaks cause damage to objectives and poor image quality (mixes with oil).

How to do a leak test:

1. Mount coverslip in chamber
2. Add media
3. Clean bottom of coverslip with ethanol
4. Place on clean Kim wipe for 30 seconds
5. Check for any media leakage
6. Remount if necessary

6. DATA REPORTING COMPLIANCE

Experimental Protocol

- Provide a detailed experimental protocol including catalog and lot numbers of reagents used
- Do not refer to protocols from past papers

Image Display

- State the display adjustments (min, max, LUT, gamma) used for each image data set
- Apply the same display adjustments to images within the same experimental data set
- Follow all journal requirements for creating figures

Instrument Information and Acquisition Settings

Specifications of your imaging system and acquisition settings MUST be reported.

- Parameters are saved in the metadata

Use the MicCheck website to determine what information you need to report.

See the papers below for best practices in data reporting and display.

- Llopis et.al. Nature Methods (2021) 18: 1463–1476.
- Jambor et.al. PLOS Biology (2021) 1161: March 31.
- Heddleston et.al. Journal of Cell Science (2021) 134: jcs254144.

Analysis Workflow

A description of analysis software (including version) and all processing steps MUST be reported

- Licensed software will have methods to export the processing steps

If using open source software (e.g. FiJi) include workflow steps and code

- Don't forget to cite both FiJi and source code papers

Always keep a copy of RAW unmodified data (includes metadata)

7. CONTINUED LEARNING

1. Literature
2. Microscope manufacturers have excellent online resources – e.g. Leica, Nikon, Zeiss, Olympus
3. iBiology Videos
4. Professional Organizations (e.g.. Canada Bioimaging, Bioimaging North America NEUBIAS