

mh_tools

Photomicrography Tool

User Manual
Version 2.0.0

Precision calculator for photomicrography and macro-rail focus stacking
magnification • field of view • depth of field • diffraction • effective aperture • stacking steps

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<https://anti-matter-3d.com/tools>

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1. Introduction

The mh_tools Photomicrography Tool is a precision calculator for photomicrography and macro-rail stacking workflows. It computes physical magnification, equivalent (crop-scaled) magnification, field of view, object-space pixel size, depth of field (DOF), diffraction and effective-aperture behaviour, lateral resolution, sensor-sampling adequacy, and recommended rail step sizes and frame counts.

Two optical models are supported:

- Camera lenses (macro approximation, with optional extension and pupil-magnification terms).
- Infinity microscope objectives (tube-lens scaling with NA-based diffraction + detector DOF).

Version 2.0.0 is a full rewrite on Python 3.13 + PySide6. The optical engine has been reviewed against primary references (Nikon MicroscopyU, photomacrography.net, Canon MP-E documentation) and extended with a diffraction / effective-aperture / exposure module and a resolution / Nyquist-sampling module. Every input that physically affects the result — aperture, magnification, pupil ratio, refractive index, wavelength — now feeds the relevant output.

This manual is written for high-end / technical users and focuses on what the tool is actually modelling, which inputs matter, and how to validate results. For the full set of equations, see Appendix A.

Scope: this is a calculator. It models first-order optics and standard diffraction / DOF criteria. It does not trace real lens groups, aberrations, or field curvature. Treat its outputs as well-grounded planning figures, then confirm against a stage micrometer for your specific rig.

mh_macro_tools - Photomicrography Tool v2.0.0

FileHelp

Photomicrography Tool

magnification · FoV · DOF · diffraction · focus stacking

v2.0.0

Optical Setup

Lens Type:

Camera Lens

Object / Objective Magnification:

1

Focal Length (mm):

60

Extension Distance (mm):

F-stop (lens):

2.8

Pupil Magnification:

1.0

Numerical Aperture (NA):

0.25

Tube Lens Focal Length (mm):

200

Refractive Index n (1.0 air / 1.515 oil):

1.0

Raynox Lens:

DCR-150

Raynox Use Mode:

As Tube Lens

Camera Mount:

Nikon F

Mount Register (mm):

46.5

Sensor & Detector

Sensor Width (mm):

23.5

Sensor Height (mm):

15.7

Crop Factor:

1.5

Pixel Size (µm):

4.22

Wavelength (nm):

550

CoC Factor (× pixel):

2.0

Diffraction Criterion:

Standard (CoC)

Resolution Width (px):

5568

Resolution Height (px):

3712

Magnification

Physical Magnification:1.0000

Equivalent Mag (× crop):1.5000

Extension-based Mag (camera):-

Tube/Projection Focal Used (mm):-

Raynox Req. Mount Ext (mm):-

Raynox Ext Delta (mm):-

Field of View & Sampling

Field of View W (mm):23.5000

Field of View H (mm):15.7000

Field of View W (µm):23500.0

Field of View H (µm):15700.0

Sensor Diagonal (mm):28.262

Object Pixel Size (µm):4.2200

Pixel Coverage (px):5568 x 3712

Depth of Field & Stacking

Depth of Field (mm):0.094528

Depth of Field (µm):94.528

Focus Step Size (mm):0.066170

Focus Step Size (µm):66.17

Frames / Focus Steps:227

Effective Overlap (%):30.0

Total Focus Travel (mm):15.000

Diffraction & Exposure

Effective f-number:f/5.6

Light Loss (stops):2.00

Airy Disk Ø (µm):7.515

Diffraction:Within criterion — sharp

Diffraction Sweet Spot (f/):f/3.1

Objective Resolution (µm):-

Resolution at Sensor (µm):-

Sampling:-

M = 1.000x | DOF = 94.53 µm | step = 66.17 µm | 227 frames | overlap 30%

Within criterion — sharp

2. Installation & Launch — Windows Version

The tool is distributed as a single signed Windows executable inside a ZIP archive. No Python, runtime libraries, or additional software is required — everything is bundled inside the EXE.

Install

- Extract the ZIP to a normal writable folder (for example your Tools or Documents folder).
- Double-click `mh_Photomicrography_Tool_Win_x64_v2_0_0.exe` to launch.

Typical distribution folder contents:

```
mh_Photomicrography_Tool_Win_x64_v2_0_0.exe    <- launch this
License_Agreement.pdf
mh_Photomicrography_Tool_User_Manual.pdf
README.txt
```

 **Tip:** No internet connection is required to run the tool. Preferences are written to `%APPDATA%\mh_tools\` on first run.

 **SmartScreen:** on first launch Windows may show a SmartScreen prompt for a newly seen signed binary. Choose More info → Run anyway. The executable is code-signed; reputation builds up over time.

3. Quick Start (most common workflow)

Default infinity-objective workflow (Nikon CFI-style 200 mm reference, PB-6 bellows, Raynox DCR-150 acting as the tube / projection lens):

- Set Lens Type to Microscope Objective (Infinity).
- Enter Objective Magnification (e.g. 10 for a 10× objective).
- Set Numerical Aperture (NA) to the objective NA (e.g. 0.25).
- Set Tube Lens Focal Length to 200 mm (or leave the reference default if you use a real 200 mm tube lens).
- Choose Raynox Lens = DCR-150 and Raynox Role = As Tube Lens if the Raynox is acting as the tube / projection lens.
- Confirm camera / sensor parameters (mount, sensor width / height, crop factor, pixel pitch, resolution).
- Set Refractive Index (1.0 for air / dry objectives; 1.515 for oil immersion) and Wavelength (550 nm green is the usual reference).
- In Macro Rail Parameters, choose overlap (e.g. 30%) and object depth / scale (e.g. 3 mm).
- Click Calculate.

The Results panel updates with magnification, field of view, DOF, diffraction / effective-aperture figures, resolution and sampling verdicts, and the recommended rail step and frame count.

 **Tip:** For a camera-lens rig (e.g. Canon MP-E 65 mm), switch Lens Type to Camera Lens, enter base magnification and f-number, and the tool will report the effective aperture and the light loss in stops at that magnification.

4. GUI Overview

The window is split into two scrollable columns:

- Left: Inputs, grouped into Magnification Parameters and Macro Rail Parameters.
- Right: Results & Recommendations (computed outputs), grouped by topic.

Mode-dependent fields

- In objective mode the Objective f-stop field is disabled, because objectives are specified by NA, not f-number.
- In camera-lens mode NA is ignored; DOF uses the macro approximation with f-number and the CoC criterion.
- Selecting a camera mount auto-fills its flange (register) distance; choosing Custom enables manual register entry.

Menus

- File: Load Profile, Save Profile, Save Results, Exit.
- Help: User Manual (opens this PDF) and About (version, licence, links).
- Auto-calculate (button row, on by default): recalculates live as you change any value. Turn it off to calculate only on the Calculate button.

[SCREENSHOT] main window in objective mode, showing the two-column input / results layout.

5. Magnification & Optical Model

5.1 Physical vs equivalent magnification

Physical magnification is the actual magnification at the sensor plane. It drives every physical output: field of view, pixel scale, DOF, diffraction, and step size.

Equivalent magnification is a display-only framing comparison, computed as physical magnification × crop factor. Crop factor does not change the physics at the sensor; it only changes framing relative to full-frame.

5.2 Infinity objectives: tube-lens scaling

Infinity objectives are designed for a reference tube-lens focal length f_{ref} (commonly 200 mm for Nikon CFI and many Mitutoyo systems). For an objective labelled M_{obj} used with an actual tube / projection lens of focal length f_{tube} :

$$M = M_{\text{obj}} * (f_{\text{tube}} / f_{\text{ref}})$$

So a nominal 10× objective on a 208.33 mm tube lens (a DCR-150 acting as the tube lens) yields a physical magnification of about 10.42×, not exactly 10×.

5.3 Camera lenses: extension approximation

For camera-lens macro work the tool can add a first-order extension term:

$$M \sim M_{\text{in}} + (E / f)$$

where E is extension and f is focal length. Real lenses deviate as focus shifts internal groups, so treat this as a practical approximation rather than an exact figure.

6. Raynox in Infinity-Objective Rigs

Raynox close-up lenses (DCR-150 / DCR-250) are specified in diopters D. A useful approximation for effective focal length is $f_{\text{raynox}}(\text{mm}) \sim 1000 / D$, giving:

- DCR-150 (~ 4.8 D) $\rightarrow f_{\text{raynox}} \sim 208.33$ mm
- DCR-250 (~ 8 D) $\rightarrow f_{\text{raynox}} \sim 125.00$ mm

6.1 Two real-world roles

A) As the tube / projection lens (modelled). When you select Raynox Role = As Tube Lens, the tool sets $f_{\text{tube}} = f_{\text{raynox}}$ in the tube-lens scaling equation. This directly changes physical magnification.

B) As an additional relay / quality element (not modelled as magnification). If you already have a defined tube-lens focal length (e.g. a real 200 mm tube lens), adding a Raynox can improve correction. Any magnification change then becomes spacing-dependent and is not stable without measuring the exact geometry, so the tool does not model it.

6.2 Bellows / extension guidance

When the Raynox acts as the tube lens, practical assemblies place it roughly one focal length from the sensor. The tool reports:

- Raynox Required Mount Extension (mm): a first-order placement guideline, f_{raynox} minus the mount register distance.
- Raynox Extension Delta (mm): your entered extension minus that guideline.

Focus helper, not magnification: these two figures help you place the Raynox at focus. They do not change the magnification, which is governed by the tube-lens relationship above.

7. Focus Stacking

Focus stacking captures a sequence of frames at stepped focus distances and merges the sharp zones. The tool links four quantities — depth of field, step size, frame count, and effective overlap — and lets any one of them drive the others. It supports automated rails, manual focus-by-hand, and single-shot planning.

7.1 The governing relationships

The depth of field per frame is the in-focus slab thickness. To avoid gaps between slices the focus step must be no larger than the DOF; the overlap fraction sets the safety margin:

```
step (mm)      = DOF (mm) * (1 - overlap)
frames         = ceil(object depth / step)
effective overlap = 1 - step / DOF
```

These are algebraically reversible, which is what makes the calculation work in any direction. Given DOF, fixing any one of {overlap, step, frames} determines the other two.

7.2 Drive Stacking By — DOF / Step / Frames

DOF (auto). You set the overlap; the tool derives $\text{step} = \text{DOF} (1 - \text{overlap})$ and the frame count. This is the classic planning direction.

Step Size. You set the physical step (e.g. your rail's smallest reliable increment, or a manual-focus increment). The tool computes the frames needed and the effective overlap you are actually getting. If the step exceeds DOF the effective overlap goes negative — a flagged warning that focus gaps will appear.

Frame Count. You set how many frames you are willing to shoot; the tool divides the object depth into that many equal steps and reports the resulting step and effective overlap, so you can see whether your chosen count is sufficient.

Effective overlap: this is the honest overlap your settings produce, which may differ from the overlap field when a step or frame count drives the calculation. Negative values mean $\text{step} > \text{DOF}$, i.e. unsampled gaps between in-focus slabs.

7.3 Stacking Mode

- **Motorised Rail** — the step is the rail increment and the frame count is the number of captures.
- **Manual Focus Steps** — no rail; the same numbers describe how many focus-ring adjustments to make and the depth each advances. Useful for a larger subject shot by hand with a 1:1 macro and a handful of refocuses.
- **Single Shot** — no stacking; the tool reports DOF only (one frame). Use it to check whether a subject fits within a single frame's depth of field at a given aperture.

7.4 Working interactively

With Auto-calculate on (the default), every change recalculates immediately, and the computed step and frame count are written back into the Step Size and Frame Count fields. This keeps the Focus Stacking section live: tweak overlap, f-stop or object depth and watch the step settle to a value that suits your rig and the time you have. When you find a step you like, switch Drive Stacking By to Step Size — the field already holds that value,

ready to fine-tune — and the frame count follows. Turn Auto-calculate off to calculate only on the Calculate button.

7.5 Which DOF model is used

- Objective mode: DOF combines a diffraction (wave-optical) term and a detector (CoC) term, scaled by the imaging-medium refractive index. See Appendix A.
- Camera mode: DOF uses the macro approximation at the effective aperture with the CoC criterion, including the pupil-magnification correction.

 **High NA:** objective DOF becomes extremely shallow at high NA — a few microns or less. If the frame count looks extreme, that is physically correct, not a bug. Confirm overlap, pixel pitch, CoC factor and NA before doubting the number.

8. Diffraction, Effective Aperture & Exposure

At macro and micro magnifications the working aperture is not the marked f-number. As magnification rises, the effective aperture grows, diffraction softens fine detail, and you lose light. This section covers the outputs that quantify all three, and the criterion that decides when diffraction is worth worrying about.

8.1 Effective f-number

For a camera lens the effective (working) f-number at magnification M is:

$$N_{\text{eff}} = N * (1 + M / p)$$

where N is the marked f-number and p is the pupil magnification (exit / entrance pupil ratio; 1.0 is a safe default for symmetric macro lenses). At 1:1 with p = 1 the effective aperture is two stops slower than marked; at 3:1 it is four stops slower.

8.2 Light loss in stops

The exposure penalty from that effective-aperture change is:

$$\text{Light loss (stops)} = 2 * \log_2(1 + M / p)$$

For example a Canon MP-E 65 mm at 3:1 and f/8 reports an effective aperture of f/32 and a light loss of 4.00 stops — matching the lens documentation.

8.3 Airy disk

Diffraction spreads a point into an Airy disk whose diameter at the sensor is:

$$\text{Airy (um)} = 2.44 * \lambda * N_{\text{eff}} \quad (\text{camera})$$

Because N_eff grows with magnification, the Airy disk grows too. This is why every high-magnification macro setup eventually becomes diffraction-influenced — it is physics, not a lens or app fault.

8.4 The diffraction criterion (Strict / Standard / Practical)

Whether a given Airy disk "matters" depends on the sharpness standard you hold the image to. The tool computes the Airy disk exactly, then compares it against a threshold you choose. Only the threshold and the wording change — never the underlying number.

Criterion	Threshold and intended use
Strict (pixel)	Threshold = 1 pixel pitch. Pixel-level pixel-peeping; the most demanding standard. Almost any macro aperture will read as softened.
Standard (CoC)	Threshold = circle of confusion (CoC factor x pixel). A balanced default that reflects normal output sharpness.
Practical (relaxed)	Threshold = 2 x CoC. Assumes focus stacking plus capture sharpening (deconvolution / high-pass) recover fine detail — which in practice they do.

The verdict is then reported in three neutral zones relative to the chosen threshold T:

Verdict	Condition
Within criterion — sharp	Airy <= T. Diffraction is below your chosen standard.
Mild diffraction softening (recoverable)	T < Airy <= 1.5 T. Slight softening, normally recovered by stacking and sharpening.
Diffraction-limited at this aperture	Airy > 1.5 T. Diffraction dominates; opening up would help if depth of field allows.

 **Tip:** A standard 1:1 lens at f/4 (effective f/8) reads "mild softening (recoverable)" under the Standard criterion and "within criterion" under Practical — not a defect, just the optics. Choose the criterion that matches how much you rely on post-processing.

8.5 Diffraction sweet spot

In camera mode the tool also reports the marked f-number whose effective Airy disk exactly meets the criterion:

$$N_{\text{eff}}^* = T / (2.44 * \lambda)$$

$$\text{sweet spot (marked f/)} = N_{\text{eff}}^* / (1 + M / p)$$

Shooting at or wider than this marked aperture keeps diffraction within your standard. Combined with the DOF figure, it brackets the usable aperture range: open enough to satisfy the sweet spot, closed enough to cover the depth you need per frame — and let stacking bridge the rest.

8.6 Refractive index & wavelength

The Refractive Index input (n) scales objective DOF: 1.0 for dry / air objectives, 1.515 for oil immersion. Wavelength sets the diffraction and resolution reference; 550 nm (green) is the usual default. For UV or IR work set the actual capture band — shorter wavelengths resolve finer and diffract less, longer wavelengths the reverse.

9. Resolution & Sensor Sampling (Nyquist)

For objective rigs the tool reports the optical resolution limit and whether your sensor samples it adequately.

9.1 Lateral resolution (Rayleigh)

Specimen-side resolution (um) = $0.61 * \lambda / NA$
Sensor-side projection (um) = $M * \text{specimen-side resolution}$

The specimen-side figure is the smallest feature the objective can resolve on the subject. The sensor-side figure is that feature projected onto the sensor at the current magnification — which is what your pixels must capture.

9.2 Nyquist sampling verdict

To capture the resolved detail without losing it, a pixel should be no larger than half the sensor-side resolved feature. The tool reports:

Verdict	Meaning
Well sampled (Nyquist OK)	Pixel <= half the resolved feature — the sensor captures the optical detail.
Marginal sampling	Pixel between half and one resolved feature — some detail is at risk.
Undersampled (detail lost)	Pixel larger than the resolved feature — the sensor is the bottleneck.

Empty matching: if the verdict says undersampled, more magnification (a longer tube lens or a higher Raynox) or a finer-pitch sensor will recover detail. If it says well sampled with large margin, you may be over-magnifying and could trade some magnification for field of view and light.

10. Profiles (Load / Save)

Save Profile writes all input fields to a JSON file. Load Profile restores them. Profiles from earlier versions load cleanly; any inputs new to v2.0.0 (pupil magnification, refractive index, wavelength) simply take their defaults when absent.

Best practice

- Create one named profile per rig, e.g. D500_CFI10x_PB6_DCR150.json.
- Keep sensor parameters inside the profile so field of view and pixel scale stay consistent.
- Save a profile once you have validated a rig against a stage micrometer — it becomes your trusted reference.

11. Interpreting Results

Output	What it means
Physical / Equivalent Magnification	Actual sensor-plane magnification, and the crop-scaled framing comparison.
Tube / Projection Focal Used	The focal length that drove tube-lens scaling (real tube lens, or Raynox if set as tube lens).
Field of View (mm / um)	Sensor dimensions divided by physical magnification.
Object Pixel Size (um)	Pixel pitch divided by physical magnification — the subject area one pixel covers.
Depth of Field (mm / um)	Objective: diffraction + detector model scaled by refractive index. Camera: macro approximation at the effective aperture.
Focus Step Size / Frames / Focus Steps	The operative step and the number of frames, in whichever direction you are driving the stack.
Effective Overlap (%)	The real overlap your settings produce. Negative means step > DOF (focus gaps).
Total Focus Travel (mm)	The object depth the stack spans.
Effective f-number / Light Loss	Working aperture at this magnification and the exposure penalty in stops.
Airy Disk / Diffraction	Diffraction spot size at the sensor and its verdict against the chosen criterion.
Diffraction Sweet Spot (f/)	Marked aperture whose effective Airy just meets the criterion (camera mode).
Objective Resolution / Resolution at Sensor / Sampling	Optical resolution limit, its sensor-side projection, and the Nyquist verdict (objective mode).

Sanity checks

- Physical magnification should change when you change f_{tube} or set the Raynox as the tube lens.
- Equivalent magnification should scale linearly with crop factor.
- Field of view measured against a stage micrometer should match the calculated value within practical tolerance.

12. Troubleshooting

- Results show dashes: a required input for the selected mode is missing (NA for objectives, f-stop for camera lenses).
- Diffraction always flagged: that is the criterion, not a defect. A standard lens at 1:1 is diffraction-influenced by physics; switch the Diffraction Criterion to Standard or Practical, and read the sweet-spot aperture.
- Effective overlap is negative: your step exceeds the depth of field, so slices will have unfocused gaps. Reduce the step, add frames, or open the aperture (more DOF).
- Frame count looks extreme: at high NA or magnification, DOF is genuinely tiny. Verify overlap, pixel pitch, CoC factor and NA before doubting it.
- Step / Frame driver seems ignored: the matching field was left empty, so the tool fell back to the DOF-derived step (a note explains this). Enter a value or switch the driver to DOF (auto).
- Unexpected objective magnification: check whether the Raynox is set as the tube lens, and confirm the tube-lens focal length.

13. Best-practice Workflows

 **Tip:** Treat this tool as a rig-validation calculator: plan with it, then confirm the critical numbers physically.

- Measure / confirm sensor parameters once (width, height, pixel pitch, resolution).
- Build a profile for each optical stack.
- Do a field-of-view sanity check with a stage micrometer; calculated and measured FoV should agree within practical tolerance.
- Find the diffraction sweet spot using the verdict before committing to an aperture.
- For stacking, start conservative (higher overlap), then optimise once rail repeatability and subject stability are confirmed.

14. Licensing

This software is licensed under Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0).

For a plain-English summary of permitted use and restrictions, see License_Agreement.pdf, included in the distribution.

Contact: questions and permission requests via <https://anti-matter-3d.com/contact/>

15. Changelog (Summary)

- v2.0.0 — Full rewrite on Python 3.13 + PySide6 (dark-amber theme). New diffraction / effective-aperture / exposure module (effective f-number, light loss in stops, Airy disk). Selectable diffraction criterion (Strict / Standard / Practical) with neutral, criterion-relative wording and a suggested sweet-spot aperture. Bidirectional focus-stacking engine — drive by DOF, step size or frame count, with effective-overlap read-out, live Auto-calculate, and step/frame autofill. Stacking modes: Motorised Rail, Manual Focus Steps, Single Shot. New resolution / Nyquist-sampling module. Added refractive-index, wavelength and pupil-magnification inputs; pupil correction applied to camera DOF and effective aperture. Per-parameter tooltips, wheel-safe dropdowns, and a Help → User Manual link. Optical engine reviewed against primary references. Mount register auto-fill. Backward-compatible profiles.
- v1.3.4 — Results panel moved right for 1080p usability; refined window sizing; stable Nikon CFI 200 mm + DCR-150 default profile; objective f-stop disabled in objective mode; Raynox extension guidance aligned with tube assemblies.

Appendix A: Calculation Equations

Symbols and Units

- M : **physical magnification** at the sensor (dimensionless), e.g. 1 for 1:1, 10 for 10×
- M_{equiv} : “equivalent magnification” (display only)
- M_{obj} : objective nominal magnification (e.g. 10 for 10×
- f_{ref} : objective design / reference tube lens focal length (mm), often 200 mm
- f_{tube} : tube / projection lens focal length actually used (mm)
- w_s, h_s : sensor width / height (mm)
- p : pixel pitch (μm)
- $p_{\text{mm}} = \frac{p}{1000}$: pixel pitch (mm)
- k : detector / CoC factor (dimensionless), typically $k \in [1,3]$
- $c = k p_{\text{mm}}$: detector criterion / “circle of confusion” term (mm)
- N : marked f-number (camera lens only)
- ρ : **pupil magnification** (exit / entrance pupil ratio); $\rho = 1$ for a symmetric lens
- N_{eff} : effective (working) f-number at magnification M
- NA : numerical aperture (objective only)
- n : **refractive index** of the imaging medium (1.0 air / dry, 1.515 oil)
- λ : wavelength, default $0.55 \mu\text{m} = 550 \text{ nm}$ (green reference)
- overlap : step overlap fraction (e.g. 0.30 for 30%)
- D : object depth / rail travel needed for stacking (mm)
- Crop : crop factor (dimensionless)

v2.0.0 additions: pupil magnification ρ , effective f-number N_{eff} , light loss in stops, refractive index n in objective DOF, Airy-disk diameter, Rayleigh resolution and Nyquist sampling. New terms are flagged **[v2.0.0]** below.

Magnification

Camera Lens (with optional extension approximation)

User enters base magnification M_{in} . If extension E (mm) and focal length f (mm) are provided:

$$M \approx M_{\text{in}} + \frac{E}{f}$$

Otherwise $M = M_{\text{in}}$.

Infinity Microscope Objective (tube lens scaling)

Infinity objectives are specified for a *design* tube lens focal length f_{ref} . Objective focal length $f_{\text{obj}} = f_{\text{ref}}/M_{\text{obj}}$. With an actual tube / projection lens f_{tube} :

$$M = \frac{f_{\text{tube}}}{f_{\text{obj}}} = M_{\text{obj}} \left(\frac{f_{\text{tube}}}{f_{\text{ref}}} \right)$$

Raynox as the tube / projection lens

Raynox close-up lenses are specified in diopters D (m^{-1}); effective focal length $f_{\text{raynox}}(\text{mm}) \approx 1000/D$. Acting as the tube / projection lens:

$$M = M_{\text{obj}} \left(\frac{f_{\text{raynox}}}{f_{\text{ref}}} \right)$$

Raynox spacing on a bellows / extension (focus constraint, not magnification)

Practical assemblies place the Raynox roughly one focal length from the sensor, $L_{\text{sensor} \rightarrow \text{raynox}} \approx f_{\text{raynox}}$. With mount flange (register) distance R :

$$E_{\text{req}} \approx f_{\text{raynox}} - R$$

This governs **focus / placement**; magnification is still set by the tube-lens relationship above.

Equivalent Magnification (display only)

$$M_{\text{equiv}} = M \cdot \text{Crop}$$

Note: physical calculations (FoV, pixel scale, DOF, diffraction, step size) use M and the real sensor dimensions.

Field of View (FoV)

$$\text{FoV}_w = \frac{w_s}{M} \quad \text{FoV}_h = \frac{h_s}{M}$$

In microns, $\text{FoV}_{\mu\text{m}} = 1000 \text{ FoV}_{\text{mm}}$.

Pixel Scale on the Subject

$$\text{ObjectPixelSize}_{\mu\text{m}} = \frac{p}{M}$$

Effective Aperture & Exposure [v2.0.0]

Effective (working) f-number — camera lens

$$N_{\text{eff}} = N \left(1 + \frac{M}{\rho} \right)$$

Light loss in stops

The exposure penalty from the effective-aperture change:

$$\Delta_{\text{stops}} = 2 \log_2 \left(1 + \frac{M}{\rho} \right)$$

At $M = 1, \rho = 1$: $\Delta = 2$ stops. At $M = 3, \rho = 1$: $\Delta = 4$ stops (e.g. MP-E 65 mm at 3:1, f/8 $\rightarrow$ $N_{\text{eff}} = f/32$).

Depth of Field (DOF)

Camera Lens (macro approximation, pupil-corrected) [v2.0.0]

With $c = k p_{\text{mm}}$:

$$\text{DOF}_{\text{mm}} \approx \frac{2Nc \left(1 + \frac{M}{\rho}\right)}{M^2}$$

(The classic $\frac{2Nc(M+1)}{M^2}$ is the special case $\rho = 1$.) If a rail / working f-stop is supplied it overrides the lens f-stop as N .

Infinity Objective (diffraction + detector term, medium-scaled) [v2.0.0]

With $c = k p_{\text{mm}}$ and refractive index n :

$$\text{DOF}_{\text{mm}} \approx \frac{n \lambda}{NA^2} + \frac{n c}{M NA}$$

(The $n = 1$ case recovers the v1.x air formula.)

Diffraction — Airy Disk [v2.0.0]

Camera lens (at the sensor)

$$\varnothing_{\text{Airy}} = 2.44 \lambda N_{\text{eff}}$$

Objective (projected to the sensor)

$$\varnothing_{\text{Airy}} = M \frac{1.22 \lambda}{NA}$$

Diffraction criterion and verdict [updated]

The Airy diameter above is exact. Whether it *matters* is judged against a threshold T selected by the user; only T and the wording change, never the physics. With pixel pitch p and circle of confusion $c_{\mu\text{m}} = k p$:

$$T = \begin{cases} p & \text{Strict (pixel)} \\ c_{\mu\text{m}} = k p & \text{Standard (CoC)} \\ 2 c_{\mu\text{m}} & \text{Practical (relaxed)} \end{cases}$$

The verdict is reported in three zones relative to T :

- $\varnothing_{\text{Airy}} \leq T$ — *Within criterion (sharp)*
- $T < \varnothing_{\text{Airy}} \leq 1.5 T$ — *Mild diffraction softening (recoverable)*
- $\varnothing_{\text{Airy}} > 1.5 T$ — *Diffraction-limited at this aperture*

Objectives have a fixed NA and are diffraction-limited by design, so no pass/fail verdict is shown for them — resolution and Nyquist sampling are the actionable read-outs instead.

Diffraction sweet spot (camera) [v2.0.0]

The marked f-number whose effective Airy disk exactly meets the criterion T :

$$N_{\text{eff}}^* = \frac{T}{2.44 \lambda} \quad N_{\text{marked}}^* = \frac{N_{\text{eff}}^*}{1 + M/\rho}$$

Shooting at or wider than N_{marked}^* keeps diffraction within the chosen standard.

Resolution & Sampling [v2.0.0]

Rayleigh lateral resolution (specimen side)

$$r_{\text{spec}} = \frac{0.61 \lambda}{NA}$$

Projected to the sensor

$$r_{\text{sensor}} = M r_{\text{spec}}$$

Nyquist sampling verdict

Let p be the pixel pitch. Adequate capture requires $p \leq r_{\text{sensor}}/2$:

- $p \leq \frac{r_{\text{sensor}}}{2} \rightarrow$ *Well sampled (Nyquist OK)*
- $\frac{r_{\text{sensor}}}{2} < p \leq r_{\text{sensor}} \rightarrow$ *Marginal sampling*
- $p > r_{\text{sensor}} \rightarrow$ *Undersampled (detail lost)*

Focus Stacking — Bidirectional [updated]

Depth of field, step, frame count and overlap are linked by:

$$\text{step} = \text{DOF} (1 - \text{overlap}) \quad \text{frames} = \left\lceil \frac{D}{\text{step}} \right\rceil \quad \text{overlap}_{\text{eff}} = 1 - \frac{\text{step}}{\text{DOF}}$$

These are reversible, so any one driver determines the rest:

- **DOF (auto):** user sets overlap $\rightarrow$ step = DOF(1 – overlap), then frames.
- **Step size:** user sets step $\rightarrow$ frames = $\lceil D/\text{step} \rceil$ and $\text{overlap}_{\text{eff}} = 1 - \text{step}/\text{DOF}$.
- **Frame count:** user sets frames $\rightarrow$ step = D/frames and $\text{overlap}_{\text{eff}} = 1 - \text{step}/\text{DOF}$.

A negative $\text{overlap}_{\text{eff}}$ means step $>$ DOF: the in-focus slabs do not meet and focus gaps appear.

Single Shot mode sets frames = 1 and reports DOF only; **Manual Focus Steps** uses the same maths as a rail but interprets the result as focus-ring advances.

In microns, $\text{step}_{\mu\text{m}} = 1000 \text{ step}_{\text{mm}}$.