

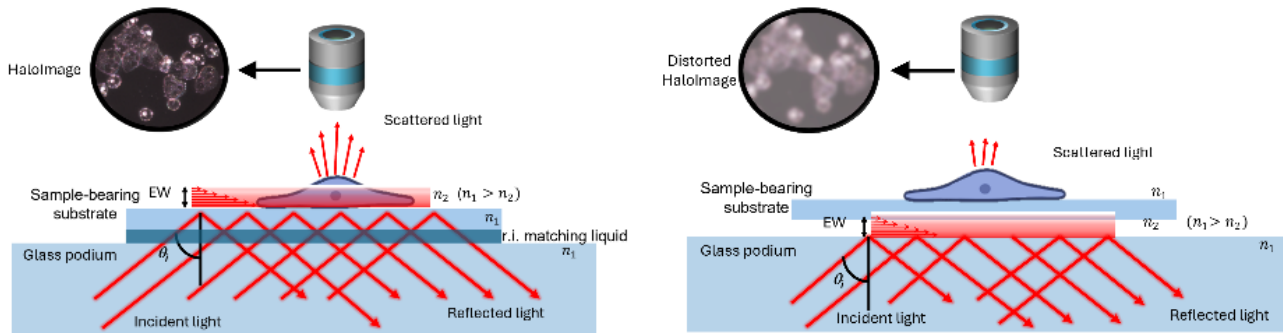
## Light Delivery Matters: Optical Coupling the Sample

When standard coverslips, glass-bottom petri dishes, silicon wafers, or other substrates are simply placed onto the round glass slide of the HaloElement, a microscopic air gap (refractive index,  $n \approx 1.00$ ) inherently forms between the two higher refractive index solids. To correctly generate and position the evanescent wave (EW) at the desired interface for optimal imaging, optical coupling via a refractive-index-matching (RIM) fluid is typically necessary.

Proper optical coupling ensures that HaloMicroscopy extracts the full detail and contrast from the sample, maintaining high resolution and eliminating artifacts caused by unwanted reflections or scattering at air interfaces.

### Generate the EW at the Right Interface

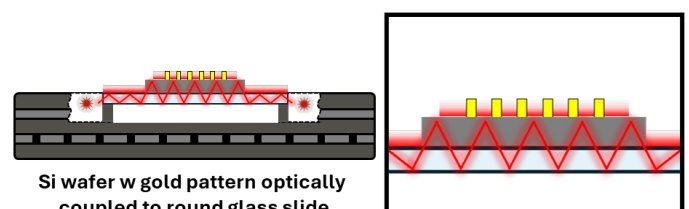
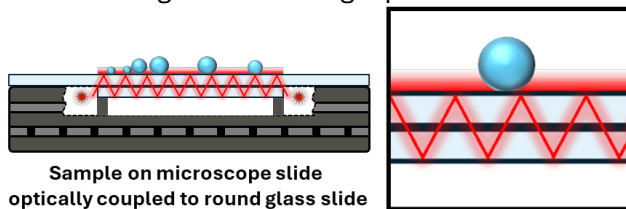
To sustain accurate EW formation and preserve image fidelity, the optical boundary between an external substrate and the glass podium must be free of any discontinuity. The figures below contrast scenarios with and without refractive-index-matching fluid, highlighting the effect on EW generation and optical continuity.



**Correct Optical Coupling Enables Proper EW Formation:** The r.i. matching liquid fills the thin gap between the external substrate and the round glass slide. This optically couples the two surfaces, allowing light to see one continuous object. The EW is generated at the solid-sample interface (be it in air or liquid), preserving image contrast and resolution.

**Air Gap Causes Misplaced EW and Image Degradation:** Without the r.i. matching liquid, an air layer remains trapped between the glass podium and the external substrate. This causes the EW to form below the coverslip, glass slide or petri dish—at the round glass slide–air interface. Depending on the thickness of the substrate, this may result in no image at all or a significantly distorted HalolImage.

Therefore, to see the structure/samples on an external surface/substrate lying on the round glass slide, optical coupling is necessary. The examples below show two cases where the evanescent wave is formed at the right interface using a r.i. matching liquid.



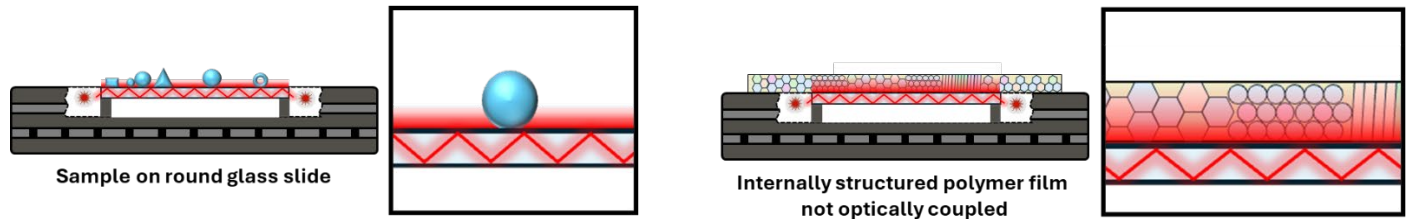
A particle sample on a standard microscope slide where the r.i. matching liquid between the wafer and the round glass slide ensures optical coupling of the standard microscope slide and the evanescent wave generated at the top of the slide scatters from the particle.

A gold nanopatterned on a silicon wafer where the r.i. matching liquid between the wafer and the round glass slide ensures optical coupling of the silicon wafer and the evanescent wave generated at the top of the wafer scatters from the nanopattern.

For detailed procedure and handling instructions, refer to Section 6.1.3 of the Installation and User Manual.

### When not to couple?

Optical coupling controls where the EW forms. To see the internal structure of an object, such as a polymer film or a banknote, the desired position of the EW is different and then a r.i. matching liquid is not used. The examples below show two cases where the evanescent wave is formed without the optical coupling including a round glass slide and an internally structured polymer film, such as a bank note.



To illuminate particle samples directly on the round glass slide, the EW needs to be formed on the round slide. For this, no r.i. matching liquid is required.

To see the structure of a complex polymer film on the round glass slide, no r.i. matching liquid is required. The evanescent wave generated the round glass slide-film interface scatters from the internal structure in the polymer film.