HaloMicroscopy: How it works

The HaloElement allows users to detect and image particles, materials, cells, bacteria, crystals, capsules, histology sections, opaque samples such as semiconductors, and polymer films, etc in air or liquids.

Key benefits of HaloMicroscopy include:

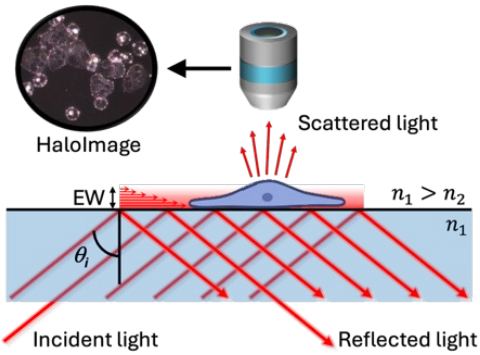
- Laser free, non-destructive method using visible light
- No labels, dyes, and stains for imaging
- Size range 100's hundreds of microns to 10's of nanometres
- Image acquisition as fast as you can take a picture
- Simple sample handling, using a glass slide, cover slip, or a petri dish in liquid or in air
- Integration with off the shelf heating stages for live cell imaging
- Standard TIFF file export compatible with industry standard image processing platforms

What is Resonance Imaging Microscopy?

Underpinned by the discovery of Resonance Imaging Microscopy at the University of Melbourne, the HaloElement employs a ring of LEDs surrounding the sample to deliver visible light from the side (rather than vertically).

Evanescent Wave (EW): It's light, but not as we know it.

The Evanescent Wave (EW) is a near-field electromagnetic field that occurs when light (in our case from LEDs) undergoes total internal reflection (TIR) at the interface between two media with different refractive indices (e.g., glass and air, or solid and liquid). Whilst the incident light is reflected, its electromagnetic field "leaks" a small distance into the second medium. This "leak" is the EW.



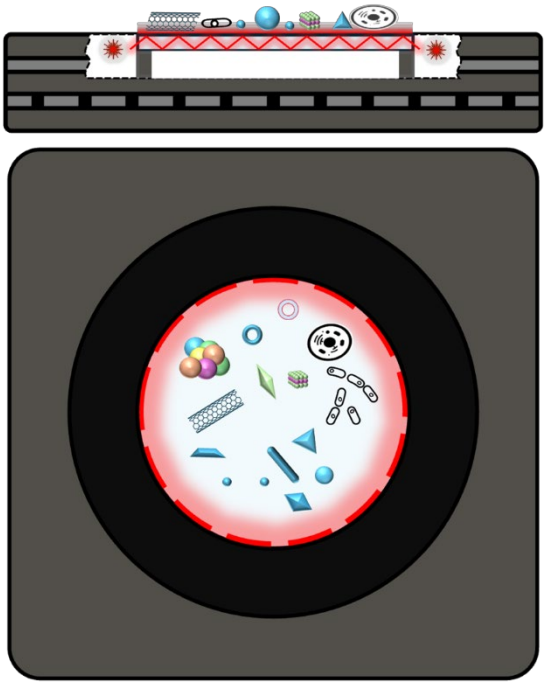
The EW 'clings' to the surface and does not propagate in the usual sense. It decays exponentially with distance from the boundary where it is formed, typically within just a few hundred nanometres. Because the EW is not a propagating wave it is therefore not constrained by the same physics that limit resolution in conventional optics, and thus it gets around the diffraction limit.

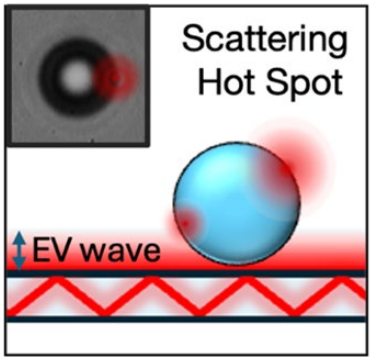
The EW can contain spatial frequencies much higher than what propagating waves can carry (*i.e.* subwavelength details). Thus, they encode information below the Abbe diffraction limit, but that information is trapped in the near field. When the EW interacts with matter, such as the sample on the slide, the EW is scattered, and visible light is produced because of that interaction. It is that visible light which is then detected by the optical microscope, using standard cameras, and is used to reconstruct the image of the sample. optical microscopes and cameras.

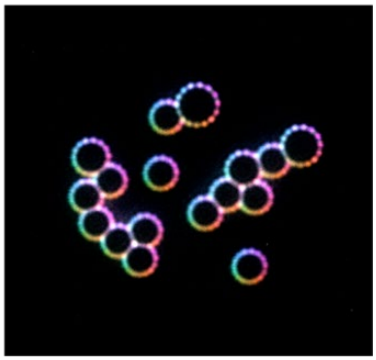
What is a Hot Spot? - Getting around the diffraction limit.

Seen most easily for simple objects of any shape, e.g. spheres, rods, prisms, etc., when the object frustrates the EW, the resulting scattering appears as edge localised hot spots when using an appropriate NA objective. Since EWs are not diffraction limited, these hot spots can be used to find the edges or detect particles well below the diffraction limit.

HaloElement Light Delivery and HaloImages







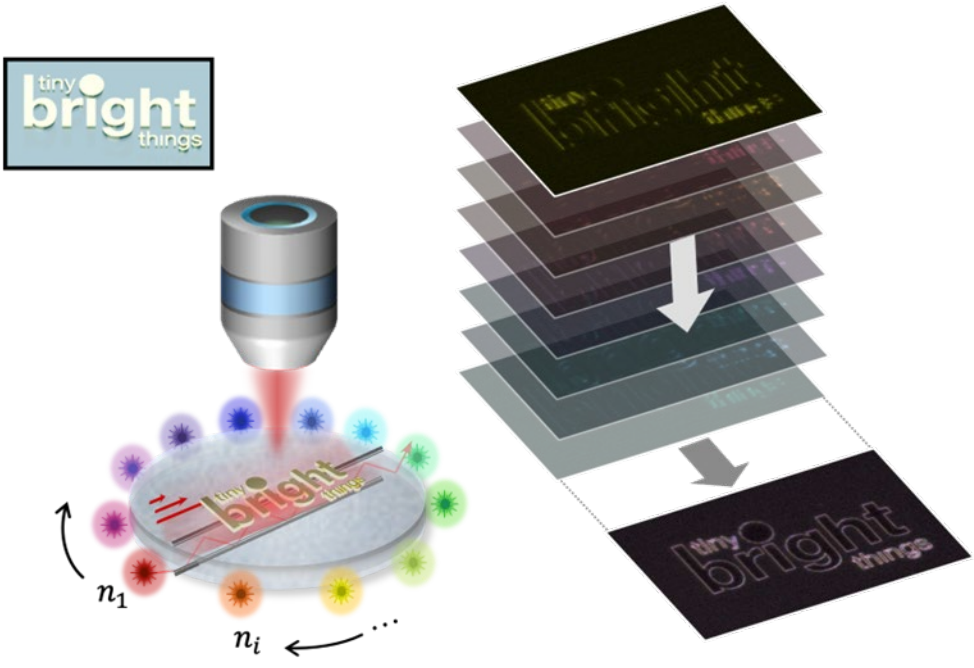
Above, Left: A particle interacting with an EW. *inset* a single hot spot on a particle edge in a brightfield microscopy image.
Above, Right: A HaloImage of 5 µm diameter polystyrene particles where the hot spots trace the edge of the particles using a faux colour scheme.

Left, Top: Side view of a HaloElement schematic with cutaway showing the delivery of the evanescent wave using LED light sources.
Left, Bottom: Top view of the schematic where the bold red lines represent the light originating from the circular array of LED sources.

What is a HaloImage? - A composite image in a few seconds.

The HaloElement creates a sequential series of 15 frames of the whole field at once. These frames are combined to form a composite image or scene called a HaloImage. A faux colour scheme is used to encode the spatial or angular orientation of each LED source with respect to the sample (like the locations on a clock face).

The example below is for a 50 nm thick gold pattern on glass fabricated using electron beam lithography.

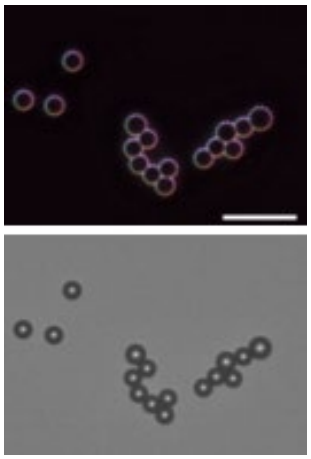


The light sources are triggered sequentially, typically using the same colour LED, but a faux colour scheme is used to encode the spatial or angular orientation of each LED source with respect to the sample. The frames are combined into a single composite HaloImage as shown on the for a 50 nm tall gold pattern deposited on glass made via electron beam lithography (rendered CAD image, upper left).

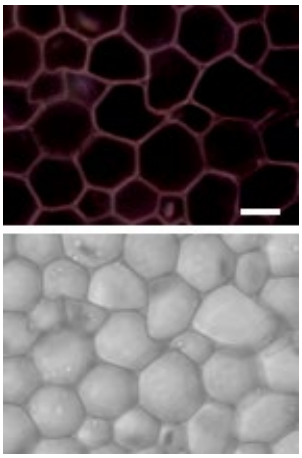
Edge Detection

The contrast in HaloMicroscopy originates, in part, from the differences in refractive index in the sample. For isotropic materials such as simple particles, isotropic films or samples with large void regions, the edges of the object will be shown in the HaloImage and not necessarily the inner region, unless there are defects in the inner region.
HaloMicroscopy detects the sample edges,

HaloImage and brightfield image for a nanofabricated pattern formed by depositing a 50 nm thick gold film. (Scale bar = 20 µm)



HaloImage and brightfield image of 5 µm polystyrene particles. The hotspots define the edges of the particles. (Scale bar = 20 µm)

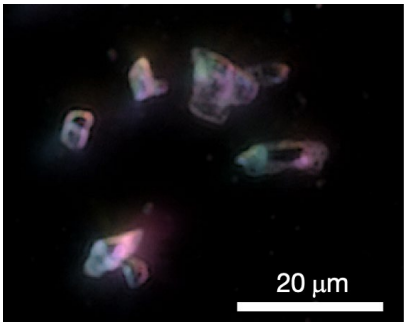


HaloImage and brightfield image of Lilium (lily) stem section where the HaloImage highlights the edges or walls of the stem. (Scale bar = 20 µm)

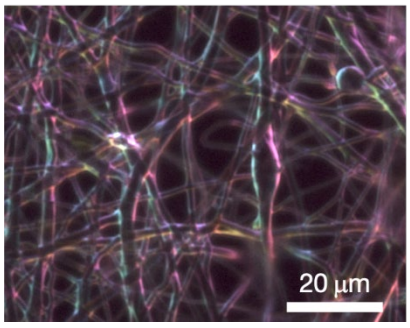
Internal Structure and Angular Information

For a complex shapes or non-isotopic materials, internal structure in a HaloImage is based on both sample geometry and local changes in the refractive index of the sample. In this case, the single frames captured from the sequential illumination from induvial LEDs captures spatial information of the sample to build a complete picture of the complex shape in the composite HaloImage. The spatial or angular position of each LED with respect to the sample is encoded in the HaloImage using a faux colour scheme (above). In a complex sample this can provide insight into 3D morphology or orientation as well as internal structure. Examples below include as crystals, fibres and embedded 2D materials in polymer films.

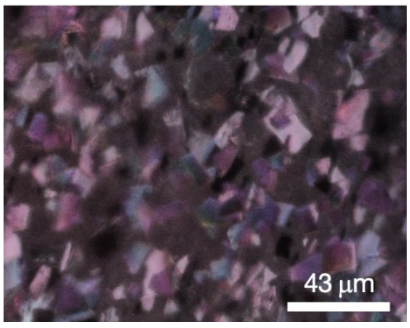
Internal Structure of Complex Samples,



Irregular shaped silicon carbide particles where the internal scattering from the HaloElement shows the internal particle structure.



Electro-spun polycaprolactone fibres. Only a portion of the 3D fibre matt interacts with the where the internal resonance illuminates the entire sample.



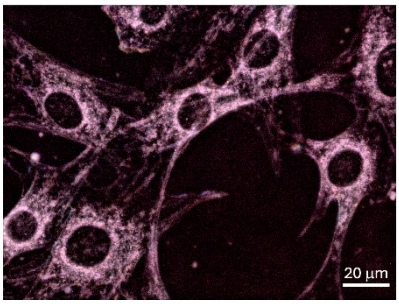
2D flakes of material embedded in a thick polymer film. The changes in colour reflect the random distribution of 3D orientations of the flakes.

See the Whole Picture

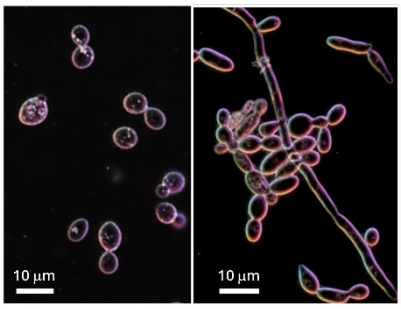
For biological samples and complex materials, a HaloImage detects both the sample edges and information on the internal properties. For example, in mammalian cells HaloMicroscopy can detect the cell membrane from a sufficient refractive index difference even though the lipid bilayer is typically 7-10 nm thick. Similarly, organelles and sub-cellular components are also detectable creating label free images of internal structure of cells, bacteria and fungi, see below.

Engineered materials can often have multiple phases, i.e. crystalline and amorphous, even for the same material. Differentiating these internal structures is often challenging using topographic measurements such as electron or atomic force microscopy. HaloMicroscopy can identify amorphous and crystalline regions based on differences in refractive index shown for a metal organic framework particle below.

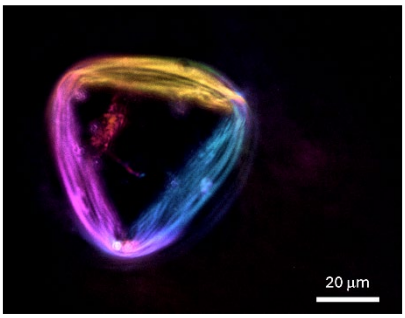
External and Internal Structure of Complex Samples



HaloImage of unstained, fixed muscle cells showing the cell membrane and the internal components including nuclei and actin fibres.



Left: Live yeast cells in sugar media showing the fungi and internals.
Right: fixed Candida Albicans showing the edges and internal compartments in the fungi.



A Metal Organic Framework particle with amorphous and crystalline regions showing different scattering behaviour.