# Imaging beyond the diffraction limit – Prospects for NIR

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## Introduction

Spectral imaging is diffraction limited and therefore features smaller than half a wavelength cannot be resolved. Nevertheless the life sciences and materials research demand laterally high-resolved insights with chemical information below the resolution limit of light.[1] A special approach to circumvent the diffraction limit is super-resolution microscopy. For example, the stimulated emission depletion microscope (STED) creates nearly spherical focal spots of 40–45 nm (λ /16) in diameter.[2] Major drawbacks of this technique are the restriction to fluorescence and the need for excitation intensities in the order of 1000 to 30000 solar units (which limits cell viability).

Another approach takes advantage of scanning probe microscopy (SPM).[3-4] The combination of SPM and near-field optical microscopy allows characterisation of the morphology and the chemistry of surfaces and cell structures beyond the diffraction limit and is called scanning near-field optical microscopy (SNOM). Common aperture-based techniques to overcome the diffraction limit employ a tiny aperture to create an evanescent field around the pinhole but these show a low S/N ratio. Aperture-limited probes are metalised glass fibre tips or hollow silicon pyramids, both with nano-fabricated pinholes. A scattering probe consists of a sharp metallic tip with a typical end diameter of 10–20 nm. The scattering probe type involves nanoscopic localisation and field enhancement of the electromagnetic radiation by scattering of the light from a metallic nanostructure.[5] Figure 1 shows examples of aperture-limited probes, scattering probes and dielectric probes.

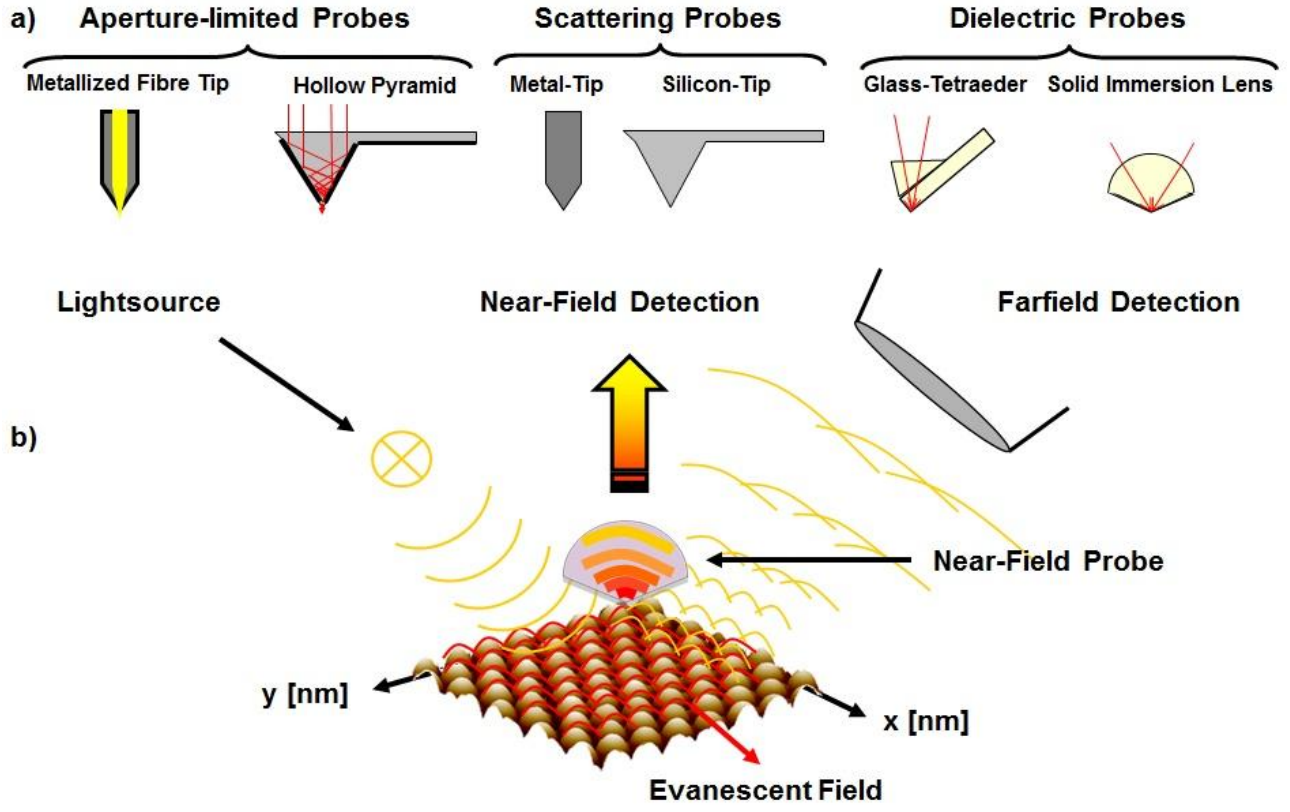


**Figure 1.** a) Examples of near-field probes: aperture-limited probes, scattering probes and dielectric probes. b) Near-field concept using a SIL as a nanoprobe for the conversion of evanescent waves to propagating waves.

An example of a dielectric near-field probe is the solid immersion lens (SIL). The SIL is attached to a micro-spectrophotometer for near-field spectroscopy. The SIL-system enhances the resolution up to λ/20 and improves the S/N ratio due to its high transmission efficiency. The SIL near-field setup has already proven its performance in the UV/Vis-range with a lateral resolution of 30 nm. A strategy and experiments are

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presented to extend the use of a SIL for NIR applications. Figure 1 also shows how the dielectric probe converts near-field information into the farfield detector: If light is coupled into a solid immersion at a certain angle, for angles larger than the critical angle evanescent components occur on the bottom tip. For incident angles smaller than the critical angle, propagating components occur that are transmitted as homogeneous fields by the SIL. The boundary between evanescent and homogeneous illumination is the angle of total reflection. The illumination point on the substrate thereby always consists of both components. The existence of a substrate under the SIL influences both components. With increasing distance from the observation level, the evanescent portion decreases. The dependencies on substrate specific parameters such as refractive index, absorption, particle size or scattering are at this point not yet sufficiently known. Scattering is described as the basic mechanism in near-field optical imaging.[6]

## Materials and Methods

### Solid immersion lens (SIL) technology

The SIL technique at Reutlingen Research Institute uses a hemispherical lens with a diameter of 1 mm and a conically-sanded tip at the bottom with a point angle of 130°. The tip forms the scanning probe and has a diameter determined to be roughly 700 nm. Depending on the wavelength range of interest, SILs of different materials are used; sapphire, cubic zirconium oxide and gallium phosphide are used for the NIR.

The SIL is mounted in a cantilever on top of the piezo scanner AS-130J of a MultiMode™ SPM from Bruker Nano Karlsruhe, Germany (Figure 2). The cantilever is fabricated of a flexible metal foil with a side mirror at the end. The SIL cantilever is placed in contact with the surface of the sample. A laser beam is directed from the side onto the cantilever mirror. The reflected light is detected by a segmented photodiode. Thus minimal movements of the SIL-cantilever in z-direction are recorded with nanometre precision for imaging the surface topography. To capture optical near-field information of the sample, the SIL unit is placed directly into the main beam of a Universal micro-spectrophotometer (UMSP 80 from Carl Zeiss Oberkochen, Germany) below the objective. The reflected light is recorded by a photomultiplier tube, a single photon counting system or by a CCD. The micro-spectrophotometer allows measurements from 200 to 2500 nm. The collection efficiency of the SNOM system is increased by a factor of 10 in comparison to standard microscopy due to the high refractive index of the SIL and the S/N is several orders of magnitude higher compared to the standard aperture limited SNOM systems.

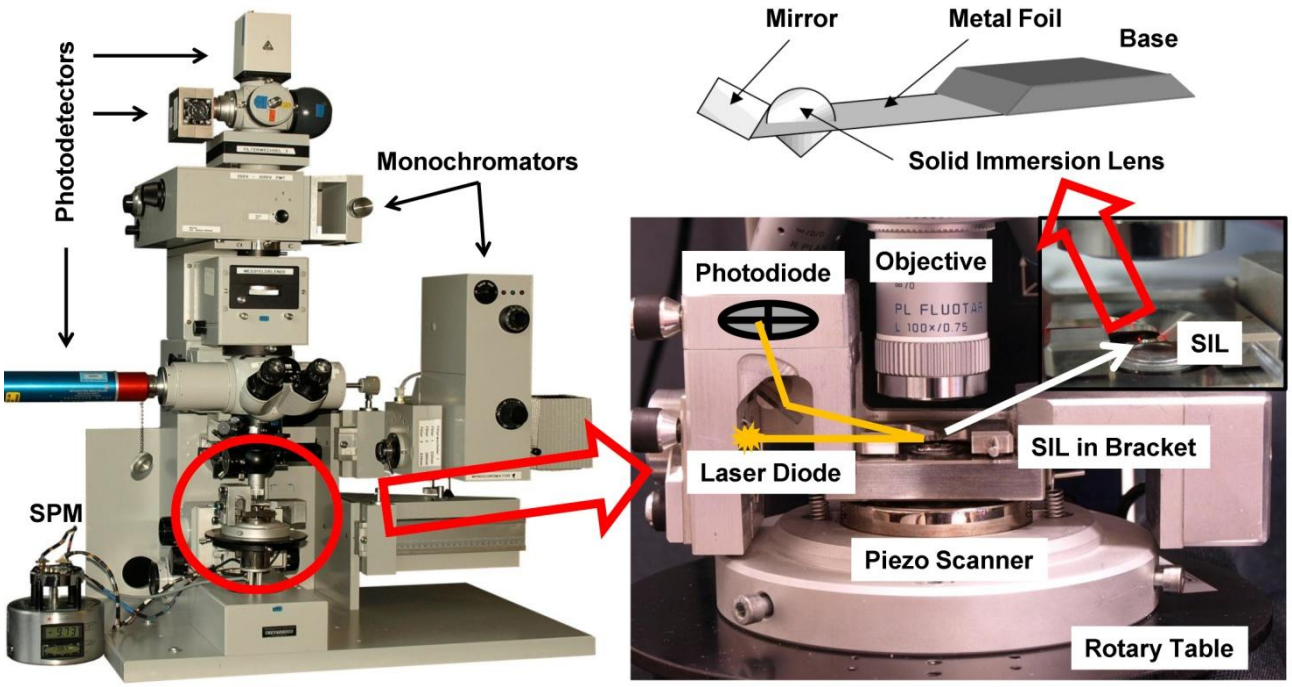


**Figure 2.** System Integration. Left: Zeiss Universal micro-spectrophotometer (UMSP 80) with SIL unit mounted underneath the objective. Right bottom: SIL cantilever on top of a piezo scanner with laser beam path for deflection measurement. Right top: SIL glued in metal foil cantilever with side mirror.

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## Results and Discussion

### Near-field imaging and near-field spectroscopy

Well-defined reference structures for spectroscopic imaging in the nanoscale are not yet available. Thus in a first experiment, the fundamental optical resolution of the system was determined to be less than 30 nm using a certified stripe pattern BAM-L002, which consisted of stripes of $Al_{0.65}Ga_{0.35}As$ in a GaAs matrix.[7] The pattern was originally developed for calibration of the length scale in scanning electron microscopy. It is also well-suited for testing the lateral resolution of SIL-SNOM-systems due to the detectable material contrast between $Al_{0.65}Ga_{0.35}As$ and GaAs. Figure 3 a) shows the layer system of the pattern and the corresponding near-field Vis image.

There are three ways in near-field microscopy to achieve different contrasts with the help of SIL technology: reflectance-SNOM (RSNOM), photon-tunneling-mode (PTM) and fluorescence-mode[8] (which will not be discussed further). In RSNOM, as depicted in Figure 1, a contrast can be produced through the creation of the near-field on a sample and subsequent conversion into propagating fields through the lens. If the SIL is illuminated with a very small luminous field diaphragm in the optical axis, then the light is refracted at the phase boundary and the substrate is illuminated. On the substrate, the light will be diffracted due to the sub-nanometre structures, and a localised near-field is created on the surface. This near-field will be converted by the interaction between the dielectric medium of the lens in propagating components and subsequently detected via the measuring field diaphragm. The detected intensity corresponds to the square of the amplitude of the propagating field. The RSNOM configuration provides the advantage that only a very small spot is illuminated and with the SIL, a very large angle of detection is available. Examples are shown in Figure 3.

The PTM is based on a different principle: a contrast can be produced through the creation of near-fields on the lens and subsequent conversion through the substrate. With this contrast method the light is coupled under the angle of total reflection into the lens and the reflected light is detected via the measuring field diaphragm. If a substrate is closer to the penetration depth of the evanescent field at the boundary surface, then photons can tunnel into the sample and thus weaken the reflected light beam. The outcome is a negative contrast. Figure 3 b) illustrates the contrast inversion on an example of a human metaphase chromosome.

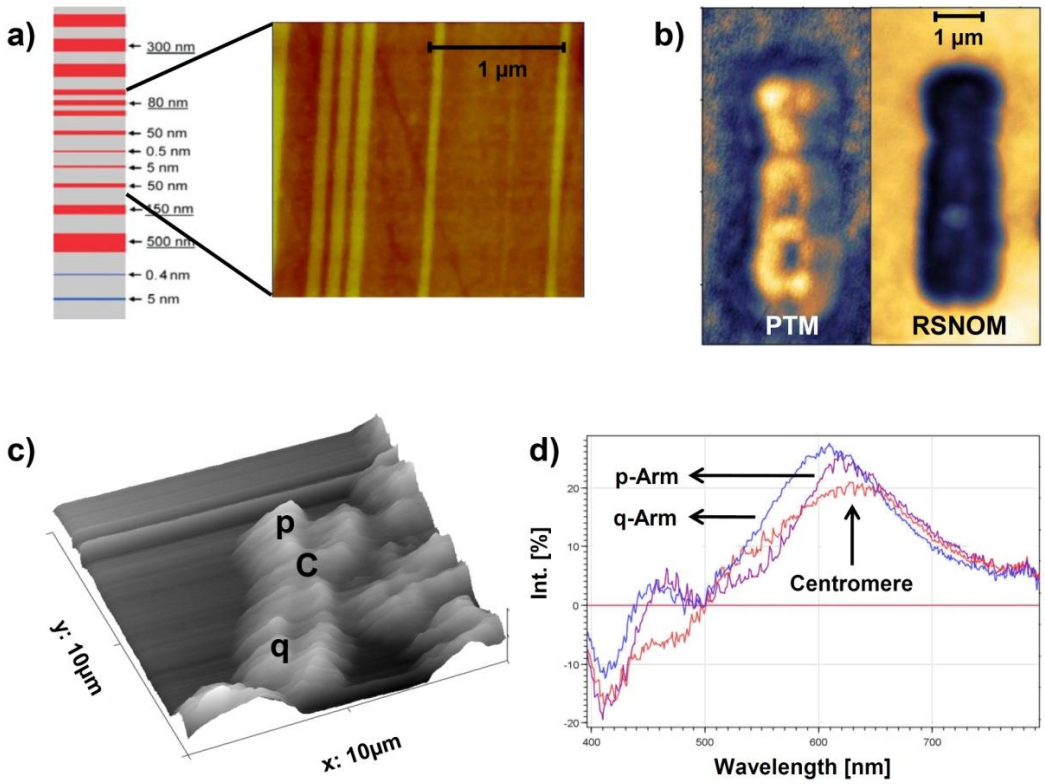


**Figure 3.** Near-field imaging and near-field spectroscopy. a) Near-field image of the certified stripe pattern BAM L002 demonstrating a lateral resolution of less than 30 nm (reflectance SNOM). Image of stripe pattern by courtesy of Bundesanstalt für Materialforschung. b) Human chromosome 10 imaged in photon-tunnelling mode (PTM) and reflectance mode (RSNOM) illustrating different contrast mechanisms. c) Near-field image of human chromosome with indication of centromere (C), p-arm (p) and q-arm (q). d) Near-field spectra of a human chromosome at the measurement positions indicated in c).

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Figure 3 c) is a three-dimensional representation of a human chromosome derived from the deflection of the SIL-cantilever. Three measuring points on the chromosome are marked with p (p-arm), C (centromere) and q (q-arm). At the indicated points, visible near-field spectra are recorded through the SIL (Figure 3 d)). The spikes in the spectra can be attributed to the morphological structure of the DNA.

## Prospects for NIR

The SIL-SNOM concept for near-field imaging and spectroscopy has already been proven for the visible wavelength range. The following considerations must be taken into account to extend the application of the SIL-SNOM to the NIR wavelength range. What relationships between scattering and absorption can be expected in terms of sensitivity? In the MIR range, absorption bands are detected which are assigned to the fundamental vibrations of functional groups in the molecule.[9] The fundamental vibrations in the MIR usually have high absorption coefficients. The absorption coefficients in the NIR show significantly lower absorbance, by at least of a factor 10 to 10,000, because of the lower cross-sections of overtones and combination bands compared to fundamental vibrations. In the SNIR (e.g. from 700 to 1200 nm) the absorption coefficients are even lower. Thus in the (S)NIR scattered light predominates over absorbed light.

Following the above considerations, we expect the spectroscopic information of near-field measurements of thin samples in the NIR range to be biased to stray light and not to absorption. Figure 4 a) and b) shows calculations of the absorption coefficient κ and scattering coefficient σ of an active pharmaceutical ingredient in an excipient matrix at 280 and 1600 nm. The calculated scattering coefficients are nearly 100 times higher than the calculated absorption coefficient at 1600 nm. Hence for NIR imaging the scattered light is predominantly responsible for image formation instead of absorption.[10]

Furthermore, NIR-cameras suffer from low S/N ratios and do not offer high resolution chips. Figure 4 c) shows the microscopic dark-field RGB-image of a glioblastoma multiforme cell (fixated with paraformaldehyde) in the visible wavelength range. This image contains straylight and not absorption information. Figure 4 d) depicts a monocyte with dendritic branches acquired in the visible wavelength range with the SIL in the near-field. Analogous to the image of the glioblastoma cell, the image formation of the monocyte is not based on absorption.

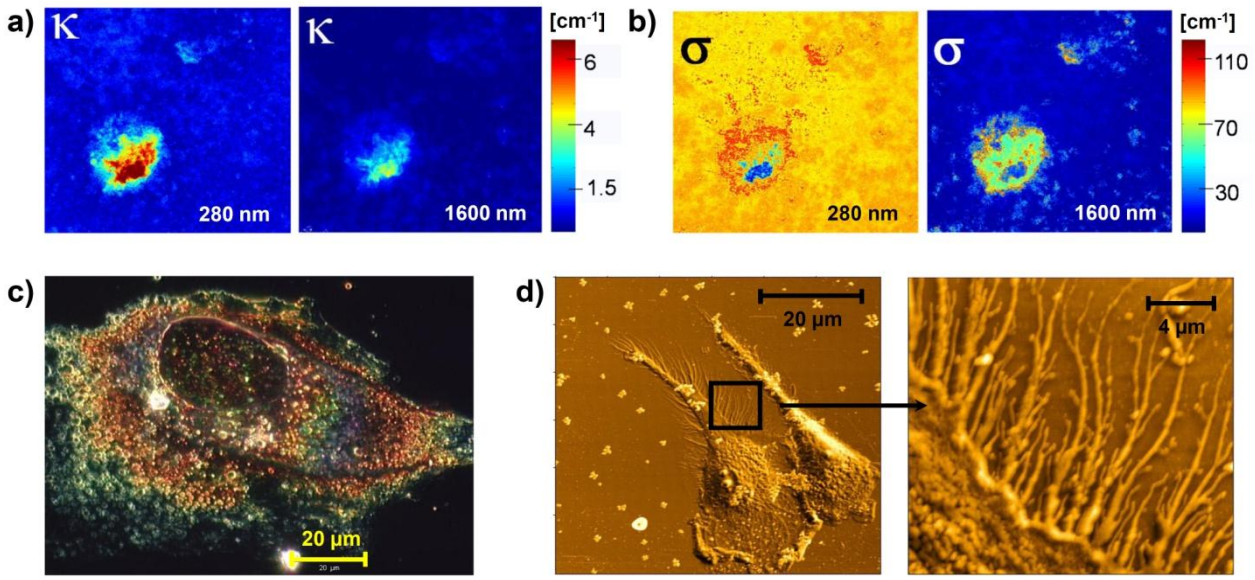


**Figure 4.** Trade-off between scatter and absorption. a) and b) calculations of absorption coefficient κ and scattering coefficient σ, respectively, at 280 and 1600 nm of an active pharmaceutical ingredient surrounded by the excipient of a tablet. c) Microscopic dark-field RGB image of a glioblastoma multiforme cell, fixed with paraformaldehyde. d) Vis-SIL near-field images of a monocyte with dendritic branches.

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As a consequence, staring imaging as well as pushbroom imaging is not suitable for a near-field NIR system. Thus only whiskbroom imaging systems meet the requirements for near-field imaging in the NIR:

- high optical throughput
- high sensitivity (high S/N), dark-field, bright-field
- fast scanning system with high lateral resolution
- multimodal spectroscopy, transmittance, reflectance
- measurement of the same sample at the same location without optical changes at different wavelengths
- no perturbation by photon diffusion with confocal setup (illumination = detection)
- all microscopy techniques optional
- high collection efficiency
- dosis limitation (to reduce photobleaching)

A new concept which integrates the above requirements is presented in Figure 5. The setup for multimodal hyperspectral near-field imaging and spectroscopy is based on a WITec imaging microscope Raman system. Spectrometers for Vis, NIR and Raman are attached via optical fibres to the microscope. The lens spectrometers are optimised for high optical throughput and spare mirrors. A piezo table enables the fast scanning for the whiskbroom imaging. The microscope integrates a near-field unit with the SIL, facilitating reflection and transmission measurements. Common microscopic contrast techniques are available. The concept additionally offers two-dimensional fluorescence and fluorescence lifetime imaging (FLIM).

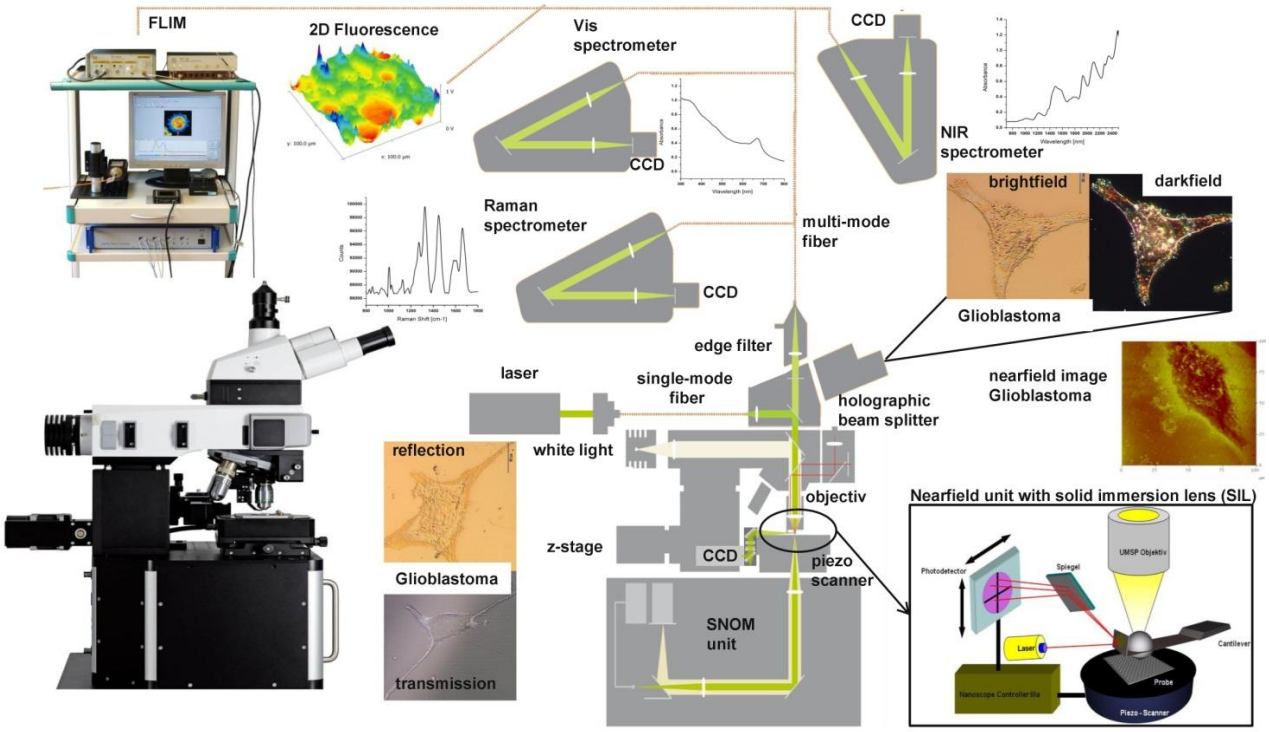


**Figure 5.** New concept for multimodal hyperspectral near-field imaging and spectroscopy: fast scanning whiskbroom imaging system on the basis of a WITec microscope with solid immersion lens for near-field measurements.

## Conclusion

The successful use of SIL for near-field imaging and spectroscopy has already been shown in the visible wavelength range. To extend its use to the (S)NIR range the low S/N in NIR and SNIR detection technology, in combination with low optical resolution and absorption coefficients, must be dealt with. We therefore propose a new concept for multimodal hyperspectral near-field imaging and spectroscopy based on whiskbroom imaging with components optimised for highest sensitivity.

Expanding the wavelength range to (S)NIR for SIL near-field imaging and spectroscopy opens up new horizons for life sciences, especially biomedicine. Additionally, the important aim of dosis limation in biomedicine will be taken into account. Materials research may benefit from concise insights below the

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diffraction limit, gathering morphological (scatter) and chemical information (absorption) at the same time. Even industries which do not mark the forefront of innovation, like food production, can take advantage of improved research methods for investigation of nanoscale components which influence composition, shelf-life, sensory and health-related aspects, resulting in a competitive advantage with monetary payoff.

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## References

1. B. Bhushan, *Scanning Probe Microscopy in Nanoscience and Nanotechnology*, Springer, Heidelberg, Germany, 1st edition (2010).
2. R. Schmidt, C.A. Wurm, S. Jakobs, J. Engelhardt, A. Egner and S.W. Hell, Spherical nanosized focal spot unravels the interior of cells", *Nature Methods* **5**, 539 - 544 (2008).
3. G. Binnig and H. Rohrer, Scanning tunneling microscopy, *Helvetica Physica Acta* **55**, 726-735 (1982).
4. R. Pool, The children of the stm, *Science* **247**, 634-636 (1990).
5. P.N. Prasad, *Nanophotonics*, Wiley-Interscience, New Jersey, USA, pp. 62-63 (2004).
6. J. Greffet and R. Carminati, *Progress in Surface Science* **56**, 133 – 237 (1997).
7. T. Merz, K. Rebner and R.W. Kessler, "Determination of the lateral resolution of a cantilever-based solid immersion lens near field microscope" in *EMC 2008* Ed by M. Luysberg, K. Tillmann and T. Weirich, **1**, 725-726, (2008).
8. T. Merz and R.W. Kessler, "Spectroscopic imaging in the near-field with an apertureless solid immersion lens microscope", in *Progress in Biomedical Optics and Imaging*, Ed by C.D. Depeursinge, SPIE proceedings **6631**, 66310V-1 - 6631V-10 (2007).
9. R.W. Kessler (ed.), *Prozessanalytik*, Wiley-VCH, Weinheim, Germany, p. 156 (2006).
10. K. Rebner, W. Kessler and R.W. Kessler, "Science-based spectral imaging: combining first principles with new technologies", in *Near Infrared Spectroscopy: Proceedings of the 14th International Conference*, Ed. by S. Saranwong, S. Kasemsumran, W. Thanapase and P. Williams, IM Publications, Chichester, UK, pp. 919-925 (2010).

Reference paper as:
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