Side-Illumination Dark Field Imaging of Immunoplasmonic Nanoprobes: From Nanophotonics to Clinical Pathology

Technology Description, Advantages, and Diagnostic Potential — Based on Plasmetrix Products & Published Literature

Plasmetrix Technologies (plasmetrix.com) has commercialized a side-illumination dark field microscopy platform that detects individual plasmonic nanoparticles on clinical specimens—enabling truly quantitative, multiplexed protein diagnostics from a simple adapter fitted to any standard pathology microscope. This technology, developed at Polytechnique Montréal's Laser Processing and Plasmonics Laboratory (LP2L) by Prof. Michel Meunier and Dr. Sergiy Patskovsky, eliminates the key optical constraints of conventional dark field condensers and opens a practical pathway to digital immunoplasmonic diagnostics in cyto- and histopathology. Backed by at least ten peer-reviewed publications (2014–2025) and now available as a commercial product line, the platform represents one of the most mature efforts to bridge nanophotonic label technology with routine clinical microscopy workflows.

How lateral RGB illumination replaces the traditional condenser

Conventional dark field microscopy relies on a specialized condenser—typically a cardioid or paraboloid type—that projects a hollow cone of light through the specimen. Only light scattered by structures enters the objective; direct illumination bypasses it. This geometry imposes a hard constraint: **the condenser numerical aperture must exceed the objective NA**, forcing pathologists to use objectives with NA below ~1.0 or to stop down high-NA oil-immersion lenses with internal iris diaphragms. The result is degraded spatial resolution precisely when it matters most—at the magnifications needed to resolve individual nanoparticles on cell membranes.

Plasmetrix's side-illumination (SI) approach takes an entirely different path (Plasmetrix Dark-Field Imaging Systems). Light from arrays of discrete-wavelength **RGB LEDs** enters the specimen laterally—from the edge of the glass slide—rather than through a substage condenser. The light propagates within the glass by total internal reflection (TIR), governed by Snell's law at the glass–air interface, until it encounters refractive index discontinuities: tissue structures, cell membranes, or—critically—plasmonic nanoparticles. At these discontinuities, light scatters upward into the collection objective. Because no illumination beam ever enters the objective's acceptance cone, **there is zero constraint on objective NA**. Any standard air objective—including high-magnification 100× dry objectives—operates at full aperture.

Important: the SI method requires air (dry) objectives and is incompatible with oil-immersion optics. The physical reason is fundamental to the operating principle. TIR-based waveguiding within the glass slide depends on the refractive index mismatch at the glass–air interface ($n_{glass} \approx 1.52$ versus $n_{air} = 1.0$). If immersion oil ($n_{oil} \approx 1.52$) is placed between the glass slide surface and the objective lens, the refractive index contrast at the upper slide surface is eliminated. TIR is destroyed, and the guided illumination light decouples from the glass waveguide and leaks directly into the objective—flooding the image with background light and eliminating the dark-field contrast that makes individual nanoparticle detection possible. **This is not a limitation in clinical practice.** Pathologists in both research and hospital settings actively prefer to avoid immersion oil, because oil contamination of glass slides—particularly irreplaceable patient specimens, archival FFPE sections, and cytology preparations—is a significant practical concern. The oil-free workflow of the SI method is therefore a clinical advantage, not a compromise.

The system operates in four steps (Figure 1). First, the rectangular or circular SI adapter delivers spectrally controlled lateral illumination from the RGB LED array. Second, metallic nanoparticles on the specimen undergo localized surface plasmon resonance (LSPR) excitation and scatter light at their characteristic resonance wavelengths—blue for silver-rich alloys, green for balanced Au/Ag compositions, red for gold-dominant particles or nanorods. Third, the microscope air objective collects only this scattered light against an intrinsically dark background. Fourth, a conventional CMOS color camera captures the image, and dedicated software decomposes the RGB channels to identify, count, and classify each nanoparticle by its spectral signature.

Product configurations and microscope compatibility

Plasmetrix offers the SI adapter in **five configurations**. For upright microscopes: (i) a fully automated software-controlled adapter for high-throughput imaging; (ii) a manual-control version for cost-sensitive labs; (iii) a specialized adapter integrated with Renishaw Raman systems combining dark field with SERS capability. For inverted platforms: (iv) a manual

adapter for Nikon microscopes; (v) a version pairing with a Prior translation stage for automated scanning of large tissue sections (product details).

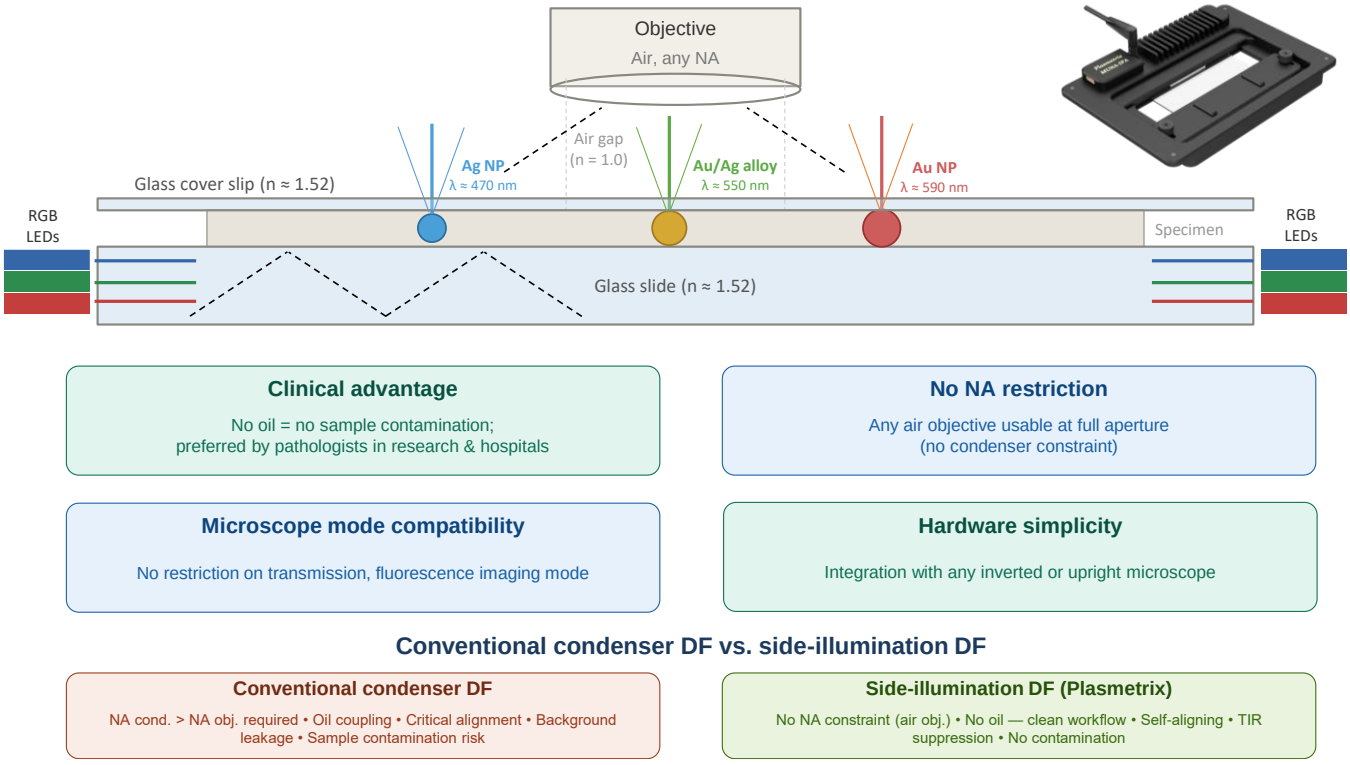

Figure 1. Side-illumination dark field microscopy: optical principle

All configurations share the same core optical design: a rectangular adapter that mounts at the specimen plane and delivers uniform lateral illumination without modifying the microscope's condenser turret, fluorescence filter cubes, or objective turret. A pathologist can switch between conventional brightfield (for H&E evaluation), fluorescence, phase contrast, and SI dark field modes on the same instrument without swapping hardware. The company describes the technology as enabling "automated multiplexed detection of plasmonic nanoparticles, digital imaging of nanoprobes and biomarkers, and spectrally (RGB) resolved dark-field microscopy."

Technical advantages over condenser-based dark field

Unrestricted objective NA (air objectives). The SI method places no constraint on the objective's numerical aperture, permitting the use of any dry objective at full aperture. Because the illumination is confined within the glass slide by TIR and never enters the objective's acceptance cone, even high-NA air objectives operate in true dark field without any condenser matching requirement. Oil-immersion objectives cannot be used, as the oil would bridge the glass–air interface and destroy the TIR waveguiding that underpins the entire method. In clinical practice this is an advantage: pathologists prefer oil-free workflows to avoid contaminating irreplaceable patient specimens.

Superior background suppression. Confining illumination within the glass slide by total internal reflection dramatically reduces tissue autoscattering and dust artifacts compared to transmission-based dark field, where residual direct light leaks through imperfect condensers.

Completely oil-free workflow. Not only is no oil coupling between condenser and slide required (as in conventional high-NA dark field), but no oil is used anywhere in the optical path. This eliminates the messiest and most time-consuming step in high-NA dark field work, prevents sample contamination, and preserves specimens for re-imaging or archival storage—a major practical advantage in hospital pathology laboratories.

Simplified alignment. Cardioid condensers are notoriously sensitive to centering and focus; the rectangular or circular SI adapter requires no critical alignment and produces uniform illumination across the field of view.

Inherent spectral selectivity. Discrete RGB LED sources enable real-time color-resolved imaging without a spectrophotometer or tunable filter, reducing both cost and complexity.

The publication record: from proof-of-concept to clinical specimens

The foundational paper by Qi, Darviot, Patskovsky and Meunier in *The Analyst* (2019) introduced cost-effective side-illumination darkfield nanoplasmonic marker microscopy (SIM), demonstrating enhanced contrast and higher resolution for individual nanoparticle detection (Analyst, 2019, 144, 1303). Nsamela Matombi et al. (2020) advanced the technology toward **fine-needle aspiration cytology**, demonstrating selective HER2 visualization on breast cancer cells using the SI adapter (ACS Appl. Nano Mater., 2020).

Marcoux-Valiquette et al. (*Cancers*, 2021) achieved the first combination of plasmonic nanoparticle bioimaging with **H&E-stained FFPE specimens**—the universal standard format in surgical pathology—simultaneously identifying four spectrally distinct nanoplasmonic markers on stained tissue sections (Cancers, 2021, 13, 3509; PMC full text). This is significant because pathologists can examine H&E morphology in brightfield and then switch to SI dark field mode on the same slide to obtain quantitative biomarker data. Zapata-Farfan et al. (2023, *Nanoscale*) extended the platform to dynamic multispectral detection of pathogenic bacteria (Nanoscale, 2023).

The most comprehensive methodological paper to date by Patskovsky, Perraton-Elorza, Largillière, Darviot, Harroun, and Meunier (*Sensors and Actuators B: Chemical*, 2025) consolidates the full pipeline from nanoparticle fabrication through digital analysis (Sens. Actuators B, 2025, 139010).

Why plasmonic nanoparticles outperform fluorescent and chromogenic labels

The physical basis lies in the extraordinary light-scattering properties of noble metal nanoparticles at their LSPR wavelength. At resonance, the scattering cross-section scales with the **sixth power of particle diameter** ($\sigma_{scat} \propto d^6$). A 60 nm gold nanosphere has a scattering cross-section of approximately 800 nm²—roughly 800 times larger than a fluorescent polystyrene bead of comparable size. Unlike fluorophores, scattering labels do not photobleach, do not saturate, and signal intensity scales linearly with illumination power.

By varying nanoparticle composition (pure Au, pure Ag, Au/Ag alloys) and shape (spheres, nanorods, bipyramids), a palette of spectrally distinct scattering colors spanning the visible spectrum is engineered. Gold nanospheres scatter green-to-orange light (LSPR ~520–560 nm); silver-rich alloys scatter blue (LSPR ~400–500 nm); gold nanorods scatter red to near-infrared via their tunable longitudinal mode (LSPR 600–1000+ nm). When conjugated to specific antibodies, these particles become **immunoplasmonic nanoprobes** that bind selectively to target antigens and are individually resolved as bright, color-coded spots against the dark background of the SI microscope.

This approach solves three persistent problems simultaneously: (i) truly **quantitative readout** through discrete digital counting of individual nanoparticles, replacing the subjective semi-quantitative scoring (0/1+/2+/3+) of conventional chromogenic IHC; (ii) **multiplexed detection of four or more biomarkers** on a single slide through spectral differentiation; (iii) **permanent label stability**—no photobleaching, no signal degradation—allowing archived slides to be re-imaged indefinitely.

Quantitative image scanning and whole-slide integration

The combination of SI dark field microscopy with motorized stage scanning (as in the Plasmetrix inverted Nikon/Prior stage configuration) enables **whole-slide imaging (WSI) in dark field mode** (Figure 2). Each field of view is captured, and software algorithms detect, count, and classify every nanoparticle by its RGB signature. Statistical analysis across thousands of fields produces a quantitative map of biomarker expression over the entire tissue section. Related work by Bhatt et al. (Scientific Reports, 2016) and the darkfield WSI adapter by Vo-Dinh and colleagues (PLOS ONE, 2013) independently validates the counting-based quantification paradigm.

The binary “yes/no” readout of individual nanoparticle detection—each particle is either present or absent in a given pixel—eliminates the analog intensity-measurement ambiguities that plague both chromogenic and fluorescence-based quantification. The 2025 Patskovsky et al. paper describes this as establishing a foundation for next-generation biosensing platforms capable of delivering precise, automated diagnostic insights.

Conclusion

Plasmetrix's side-illumination dark field microscopy platform addresses a genuine unmet need at the intersection of nanophotonics and clinical pathology. By decoupling the illumination geometry from the collection optics—delivering light laterally through the glass slide via TIR rather than through a substage condenser—the SI approach removes the single most limiting constraint of conventional dark field microscopy: the NA ceiling on objectives. The method requires air (dry) objectives, since immersion oil would bridge the glass–air interface and destroy the TIR waveguiding that produces the dark background. In clinical practice this oil-free requirement is a benefit, as pathologists in research and hospital settings prefer to avoid oil contamination of irreplaceable patient specimens.

The result is an imaging modality that can resolve and spectrally classify individual plasmonic nanoparticles on standard FFPE tissue sections and cytology preparations, is compatible with H&E staining and existing microscope hardware, and preserves specimens without contamination. The technology's maturity is evidenced by a decade-long publication arc from hyperspectral proof-of-concept (2014) through multiplexed cancer biomarker imaging on clinical-grade specimens (2021–2024) to a comprehensive instrumentation methods paper (2025). If large-scale clinical validation confirms that immunoplasmonic nanoparticle counts correlate with clinically actionable biomarker thresholds, this platform could transform protein biomarker quantification in pathology from subjective visual scoring to objective, digital, multiplexed measurement—using nothing more than a simple adapter on the microscopes pathologists already own.

Plasmetrix Technologies Inc. is a Montréal-based company originating from the LP2L laboratory at Polytechnique Montréal. Its broader product portfolio—including CORGI SPR instruments, SPECTRUNA LSPR spectrophotometers, and a range of accessories (Plasmetrix products)—positions Plasmetrix as a vertically integrated provider of plasmonic instrumentation.

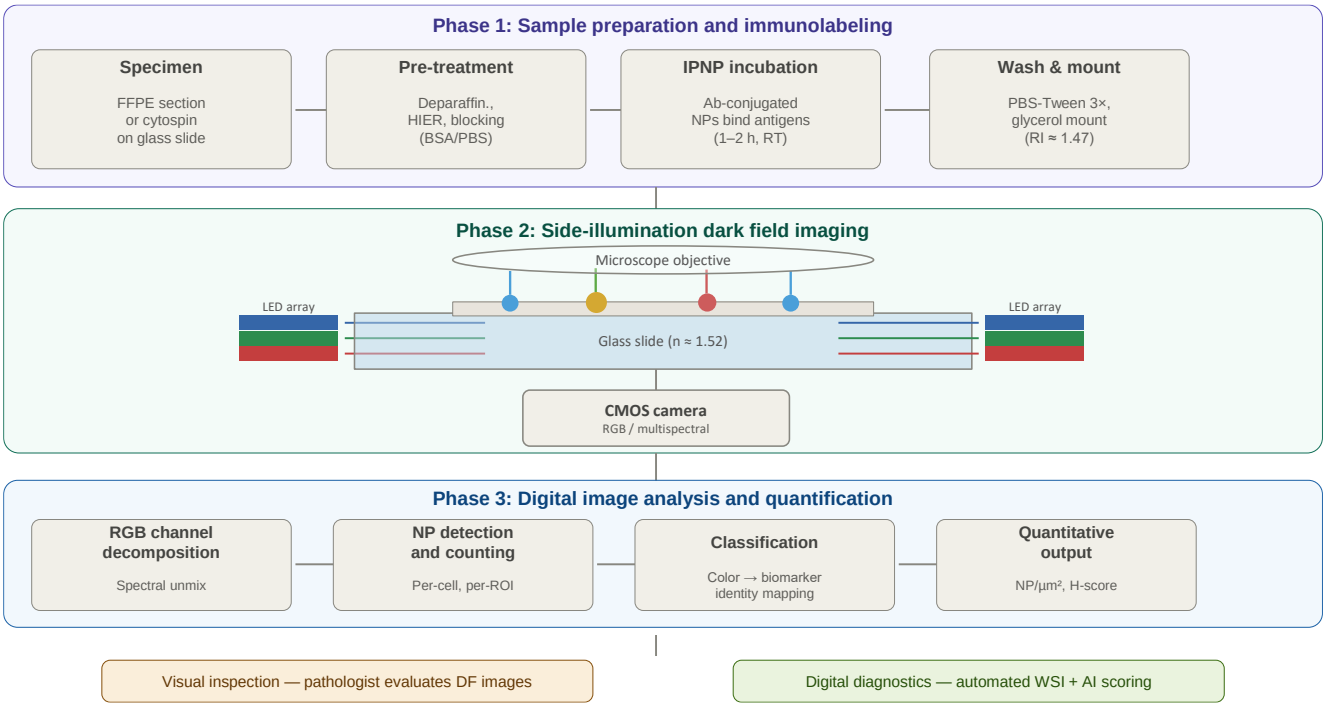

Figure 2. Immunoplasmonic nanoprobe workflow: sample to quantification.