# IRIS Kinetics Diagnostic Platform

## Ultra-sensitive and multiplexed *Digital Detection*

## Enabled by single molecule counting technology

## Introduction

Accurate, high-sensitivity detection of low-abundance biomarkers is a critical need in diagnostics. Emerging demands for point-of-care testing, early disease detection, and real-time monitoring require technologies that offer attomolar sensitivity, multiplexing, and kinetic analysis - features rarely found in a single platform.

The new frontier in biomarker analysis is single molecule counting or *digital detection*, an approach that provides resolution and sensitivity not obtainable with ensemble measurements. *Digital detection* is a disruptive technology potentially allowing most advanced disease diagnostic tools to become available at a low cost and at the point-of-need. Interferometric Reflectance Imaging Sensor (IRIS) technology meets this challenge with its unique capability to track single nanoparticle binding events across a large field-of-view, enabling label-free and digital detection modes on a single platform. This application note highlights how IRIS, using non-fluorescent labels, in particular, plasmonic gold nanorods (GNRs), enables attomolar detection of clinically relevant nucleic acids and proteins in complex media.

## Platform Overview

The IRIS platform supports two complementary modes:

- **Ensemble mode:** Label-free, real-time quantification of biomass accumulation via reflectance interferometry, and
- **Digital mode (SP-IRIS):** Single-particle enumeration using interferometric contrast of nanoparticles,

Gold nanorods serve as high-contrast scattering labels to achieve digital counting and dynamic tracking of individual binding events, even at attomolar concentrations.

## Methods

Sensor chips were coated with MCP polymer (DMA-based copolymer) to allow covalent immobilization of capture probes with high density and minimal non-specific binding. Plasmonic nanorods (~70×25 nm) were functionalized as detection labels for target microRNAs and proteins.

Digital microarray assays were run using real-time SP-IRIS tracking thousands of individual nanoparticle binding events allowing unprecedented sensitivity along with discrimination between specific target binding and high-abundance, low-affinity background molecules [1].

## Results:

### 1. MicroRNA detection with attomolar sensitivity

Utilizing digital SP-IRIS, miR-223 and miR-451a were detected with a limit of detection (LOD) below 10 aM (Fig. 1) a >100-fold improvement over conventional analog microarrays [2].

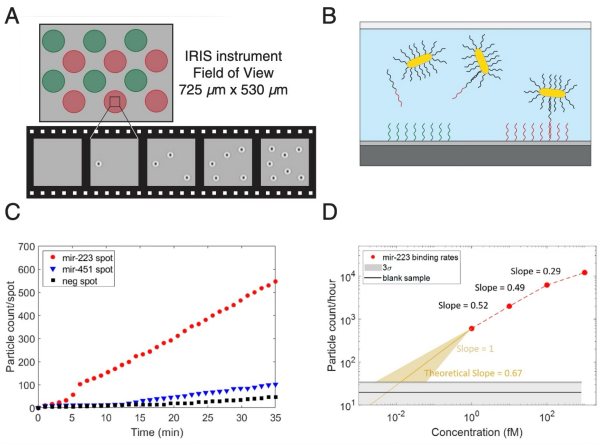



**Figure 1:** Dynamic measurements of single binding events


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**Key performance outcomes:**

- **LOD:** ~10 aM
- **Dynamic range**: 5–6 logs
- **Assay time**: <30 minutes
- **Multiplexing:** 2–10 analytes simultaneously
- **Media:** Serum, plasma, and buffer

### 2. Protein diagnostics in complex media

Protein biomarkers (e.g., allergen-specific IgE) were detected in **whole blood and serum** (Fig. 2) with minimal background interference using secondary antibody-conjugated Au nanoparticles. IRIS achieved [3]:

- Discrete single-molecule detection in unprocessed blood
- A detection limit of ~60 aM in serum and ~500 aM in whole blood.
- Nearly 6-log dynamic range with upper limit ~100 pM
- Works in biological media with no sample preparation

This approach demonstrated compatibility with **finger-prick** volumes and rapid sample-to-answer workflow.

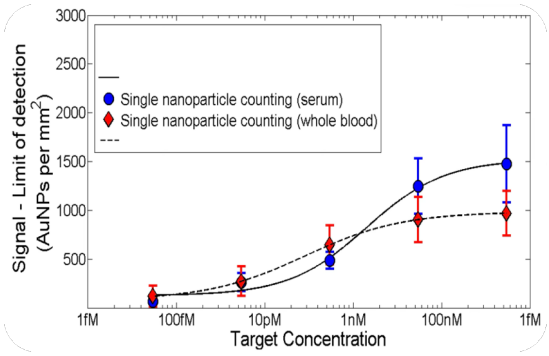



**Figure 2:** SP-IRIS single molecule counting assay for protein biomarker detection in serum and whole blood.

## Conclusion

The IRIS platform, especially when combined with nanorods-based digital detection, sets a new benchmark for molecular diagnostics. Its unique ability to measure both ensemble binding and single-molecule kinetics across a large multiplexed array unlocks early disease detection and real-time monitoring applications not accessible with legacy technologies. A recent review article authored by researchers at Roche [5] underscored IRIS as a leading photonic biosensor platform, distinguishing itself through attomolar sensitivity, multimodality, and clinical readiness.

Recent innovations in Pixel (polarization) Diversity (PD) IRIS [6] presents a paradigm change for encoding the requisite optical signature of target particles. **With PD-IRIS, single particle has become practical and available in a compact instrument.**

## Application Areas

- Early cancer diagnostics (miRNA, protein biomarkers)\
- Infectious disease screening (viral RNA, antigens)
- Allergy profiling in whole blood
- Point-of-care monitoring for cardiovascular or inflammatory conditions

## References

[1] Sevenler, D. et al. (2019) Beating the reaction limits of biosensor sensitivity with dynamic tracking of single binding events. Proc. Natl. Acad. Sci. 116, 4129–4134

[2] Kanik, F.E. et al. (2022) Attomolar sensitivity microRNA detection using real-time digital microarrays. Sci. Rep. 12, 16220

[3] Monroe, M.R. et al. (2013) Single nanoparticle detection for multiplexed protein diagnostics with attomolar sensitivity in serum and unprocessed whole blood. Anal. Chem. 85, 3698–3706

[4] Avci, O. et al. (2015) Interferometric reflectance imaging sensor (IRIS) – a platform technology for multiplexed diagnostics and digital detection. Sensors 15, 17649– 17665

[5] Aldo I. M. et al. (2025) High-performance nanobiosensing technologies for future diagnostic needs. Trends in Biotechnology

[6] Ünlü, MS et al.Pixel-Diversity Nanoparticle Detection by Interferometric Reflectance Imaging Sensor, US Patent App. 18/544,361, to be issued 2025

## Key Advantages Over Conventional Technologies

| Feature | IRIS Platform | SPR | ELISA | Digital PCR |
|---|---|---|---|---|
| Limit of Detection | **<10 aM** | ~pM | ~fM–pM | ~aM (requires amplification) |
| Multiplexing | **High** | Limited | Medium | Low |
| Label-Free | **Yes** (ensemble mode) | **Yes** | No | No |
| Digital Readout | **Yes** | No | No | **Yes** |
| Kinetic Analysis | **Yes** | **Yes** | No | No |
| Media Compatibility | **Serum, plasma, blood** | Mostly buffer | Mostly buffer | Requires purified samples |

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