Fraunhofer IPM

Single molecule detection
For point-of-care diagnosis and genotyping of bacteria

A microfluidic chip with a functionalized inner surface is inserted into the fluorescence microscope for single molecule detection.

Optical antenna assays as an alternative to PCR

Modern medicine relies on specific and targeted medication. This is essential, for instance, in the context of antibiotic resistances. Selecting the most efficient medication often requires information on a bacteria's genome. This information, however, is usually not readily available at the doctor's office. Fraunhofer IPM is collaborating with LMU Munich to develop a novel platform for pathogen detection on a single molecule level. The focus lies on an easy-to-use point-of-care (POC) detection with modest instrumentation.

Enhancing signals by means of optical antennas: A novel POC solution

Typically, target molecules are detected in vitro with the help of specific fluorescence markers. In this approach, the optical signals from these markers are enhanced by two orders of magnitude with so-called optical antennas, eliminating the need for chemical amplification via polymerase chain reaction (PCR). Antenna-enhanced single molecules can be identified using comparatively simple, low-cost instrumentation.

Optical antennas consist of nanometer-sized metal particles that concentrate light in a tiny region and also help emit light – much as macroscopic antennas do with radio waves.

The antennas are produced economically via self-assembly: A specifically designed DNA structure, a so-called DNA origami, holds the two metallic nanoparticles in place. Between these nanoparticles, the structure provides a binding site for the respective target molecule and a fluorescence marker. This patented design provides the basis for this novel assay technology.

The first assay addresses carbapenem-resistant enterobacteriaceae (CRE) via a specific DNA sequence. In principle, the single molecule assay can be adopted to molecules beyond DNA, such as RNA and antibodies as well as antigens or enzymes. A dedicated portable system for liquid handling and readout ensures a high level of automation and makes the platform suitable for POC testing.

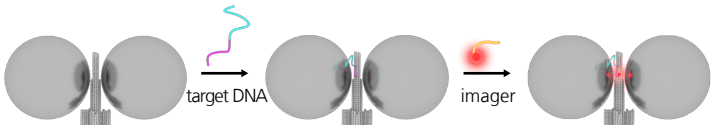
Join us as a project partner

Antenna-enhanced fluorescence detection is not yet available as a medical product.

To push the development further and eventually establish the platform as a diagnostic product, we seek for a cooperation with industrial partners.

Performance features

Fluorescence sensitivity	Single molecule sensitivity with antenna enhancement; 2.4 molecules per µm² without antenna enhancement
Imaging system	2.5 × 2.5 mm² field of view with 2.5 µm optical resolution (0.8 µm p. pixel)
Sample-to-Answer	~1 h (if target molecules are available in liquid solution)
Multiplexing	Multiplexing through spot array
Stability	Tests and reagents can be stored for several months
User interface and software	Fully automated after loading the sample



Reaction scheme for the fluorescence assay via self-assembled optical antennas. The antenna consists of a DNA pillar and two metal nanoparticles. The detection reaction takes place in the hotspot between the nanoparticles where the optical fields are enhanced by two orders of magnitude. Here, the binding site for the target DNA is located. When the target DNA has bound, the imager can attach and locate the fluorescent dye (red dot) in the hotspot of the antenna.

For POC applications, this approach is a promising alternative to conventional PCR-based methods, where the target sequence is copied multiple times to overcome the detection threshold. While PCR is an established method for central labs, it has drawbacks that impede POC use. Optical antenna assays, in contrast, rely on few binding reactions and pose little requirements on processing conditions.

Detection technology

At the heart of the processing device is a miniaturized high-resolution fluorescence microscope. Its sensitivity can compete with state-of-the-art microarray scanners or plate readers and even exceeds their resolution. A specifically developed image analysis software identifies actual single molecules, thus enabling captured target molecules count for a quantitative result. The device also features an automated fluidic system that is based on a cartridge and holds all necessary chemicals in store. The isolation of the target molecules is not yet included in the system. This remains an objective for further development.

A portable and compact system performs all reaction steps without the need for user interaction. The data is analyzed automatically and directly provides the diagnostic results. All reaction steps are carried out inside a closed cartridge.

With its small footprint, high level of automation and potentially modest cost of production, the device demonstrates its suitability for single molecule sensing in POC applications.

State of development

The underlying amplification strategy is scientifically proven, patented (DE102014105488B3, US10099195B2) and has been published in highly ranked journals, e.g. in Science 338, 506 (2012). In an ongoing project supported by the German Federal Ministry of Education and Research (grant 03VP03892), the technology is being validated.

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