Skysong Innovations
Advancing the Arizona State University Knowledge Enterprise

1475 N. Scottsdale Road, Suite 200
Scottsdale, AZ 85287-3538
Phone: 480 884 1996 Fax: 480 884 1984

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Inventors

Shaopeng Wang

Pengfei Zhang

Contact

Jovan Heusser
jovan.heusser@skysonginnovations.com

Evanescent Scattering Imaging of Single Molecules

The ability to determine molecular binding is crucial in drug screening, biomarker detection, and understanding biological processes at the molecular level. Many methods for looking at binding kinetics, such as liquid chromatography, mass spectrometry, western blot, etc., are time consuming, cause protein fragmentation/denaturation and do not allow for single molecule analyses.

Evanescent illumination is one method for detecting single molecules due to its ability to enhance light and analyte interactions. Unfortunately, current systems typically require specialized materials, including microspheres or nanomaterials, which limits their utility.

Researchers at the Biodesign Institute of Arizona State University have developed a novel imaging system that takes advantage of evanescent scattering, but can be successfully achieved on a plain glass surface. Using total internal reflection (TIR) configuration, the interference between the evanescent lights scattered by single molecules and the natural roughness of the cover glass is imaged. This imaging system can analyze molecular binding kinetics and explore the heterogeneity of single protein binding properties. It can also be used to analyze DNA conformational changes and enable detection of small biological molecules.

Potential Applications

- Biomarker detection and screening
- Drug screening
- Analyze single molecules
  - Quantify the sizes of single proteins
  - Characterize protein-antibody interactions at the single-molecule level
  - Monitor or analyze heterogeneity of single protein binding behaviors
- Track analyte axial movement with high resolution
  - Analyze DNA conformation changes to enable small molecule (e.g. microRNA) detection

Benefits and Advantages

- Label free, single molecule imaging
- Simultaneously measure size and number of individual molecules as well as analyze the kinetics of two different protein interaction processes

- No need to separate proteins or other molecules prior to detection
- Photon collection efficiency – collects only scattered light
- Multiplexed protein detection for improved screening efficiency
- Achieves 5 times larger scattering cross-section and induces less heating
- Can be integrated with fluorescent microscopy for multiplexed detection
- Automated single molecule counting with high temporal resolution
- Can recognize proteins with different molecular weights – allows for monitoring protein binding processes

For more information about this opportunity, please see

Zhang et al - Nat. Commun - 2022

Zhou et al - bioRxiv - 2022

For more information about the inventor(s) and their research, please see

Dr. Wang's Biodesign webpage

Dr. Wang's departmental webpage