Application and prospects of super-resolution fluorescence imaging for cancer screening

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Abstract. The increasing cancer incidence highlights the importance of timely diagnosis, where cancer screening plays a pivotal role. While conventional techniques like Western blot and immunohistochemistry aid detection, they are limited by optical diffraction, offering averaged expression data across cells. Super-resolution imaging has revolutionized cancer screening by enhancing resolution and revealing molecular-level assembly information. This breakthrough enables comprehensive analysis of potential cancer targets and personalized therapeutic options. This paper explores the application of super-resolution fluorescence imaging in cancer target identification, discusses mainstream techniques, and outlines future developments. These advancements promise deeper insights for cancer research and treatment, laying the groundwork for personalized medicine.

Keywords: cancer screening; super-resolution fluorescence microscopy; SMLM; biological targets.

1. Introduction

As the impact of global climate change intensifies and the demand for water resources continues to increase due to population growth, reservoirs, as important water resources control facilities, have become an important symbol of measuring the progress of a countrys water conservancy science and technology. In this context, the reservoir dispatching system based on digital technology emerged as the times require and has become a key technical support to improve the efficiency of water resources management, ensure water resources security, and meet the needs of the ecological environment [1] . Digital technology, including information collection, transmission, processing and analysis technology, provides unprecedented accuracy and response speed for reservoir dispatching. The application of these technologies not only enhances the reservoirs response ability to complex hydrological conditions, but also improves the automation level of dispatching and ensures the scientificity and real-time nature of dispatching decisions [2] . This research aims to provide a set of practical solutions for the field of reservoir dispatching and provide reference for scientific and technological development trends in related fields.

2. Introduction

The burden of cancer on global health remains significant, with it being the leading cause of death worldwide, impacting life expectancy across populations.[1] Recent data from 2020 show a substantial increase in new cancer cases, reaching 19.3 million from 14 million in 2012.

Early detection, accurate diagnosis, and timely treatment are pivotal in improving patient outcomes and quality of life.[2] Advanced imaging techniques such as optical endoscopic imaging and super-resolution fluorescence imaging play crucial roles in preclinical research and clinical applications for cancer.[3] These methods offer superior sensitivity, cost-effectiveness, and multi-scale visualization compared to traditional imaging modalities like PET, CT, and MRI. Optical imaging, in particular, provides detailed structural and functional insights into tumour tissues, aiding in precise diagnosis and treatment planning.

Previously, tumour biomarkers were primarily studied using Western blot, confocal microscopy, and immunohistochemistry, focusing on overall expression levels. [4]However, these conventional methods need to provide a high-resolution assessment of tissue structure and molecular profiles at the ultrastructural level. The spatial resolution of conventional fluorescence microscopy is limited,

hindering the assessment of intracellular features and obscuring ultrastructural information crucial to understanding the tumour process. Despite advancements, current technologies cannot offer in situ spatial assessment of functional proteomic and genomic information at the molecular or nanoscale.

The emergence of super-resolution microscopy has revolutionized cell biology research, allowing for the exploration of nanoscale cellular dynamics and complex biological processes. Specifically, single-molecule localization microscopy achieves ultra-high resolution, enabling visualization of tiny cellular structures. This technique utilizes fluorescent materials with specific properties, such as fluorescent proteins and organic fluorophores.

Single-molecule localization microscopy (SMLM) stands out among these methods for its simplicity and versatility. It shares sample preparation techniques with standard microscopy, requiring only a fluorescent dye that "flashes" after laser irradiation.[5] It allows routine immunofluorescence staining, enabling researchers to study specimens across micro, meso, and nano scales.

In medical diagnostics, imaging biological targets for cancer identification is challenging due to their small size and homogeneity. While various imaging methods have improved sensitivity and specificity, conventional light microscopy still faces resolution limitations. SMLM offers a solution by detecting biological targets at a super-resolution level, even within single cells of specific cancer cell lines.

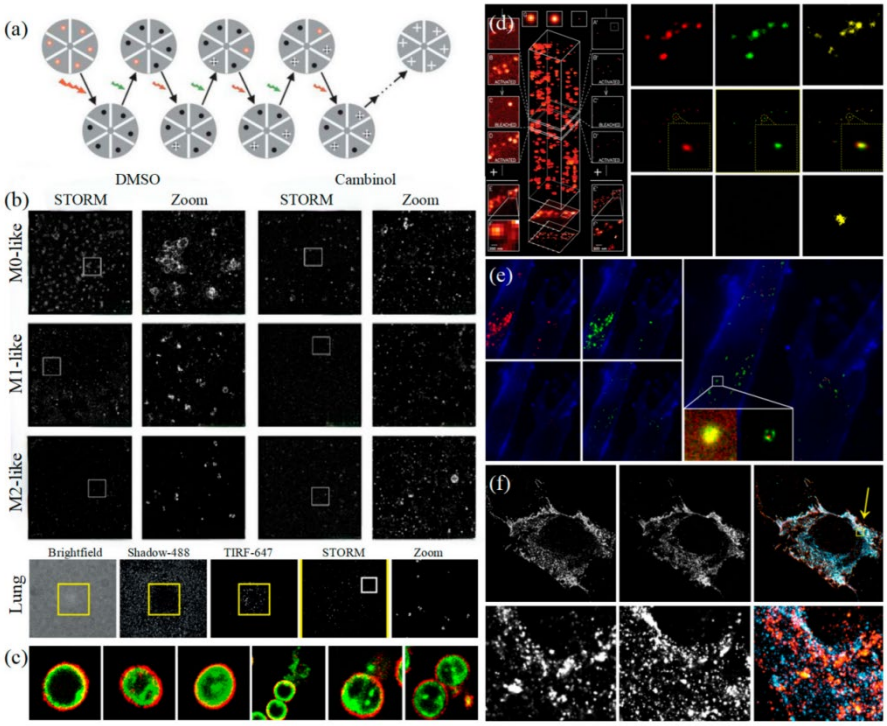

Fig. 1 Some examples of applications of super-resolution technology

Membrane receptors play a crucial role in cell communication and regulation, especially in cancer, where their aberrant expression offers potential therapeutic targets, notably for individualized immunotherapy. Super-resolution imaging techniques, like single-molecule localization microscopy (SMLM), enhance molecular visualization with unprecedented sensitivity, enabling the detection of differences in stoichiometry and expression density at 10-20 nm resolution.[6] This method has been instrumental in studying cancer-related proteins like HER2, CD19, and CX43, aiding in mechanistic understanding and observation of genomic and repair complexes in cancer cells. Furthermore, super-resolution imaging sheds light on the distribution and significance of chromatin regulatory factor clusters in cancer and cell growth. By directly quantifying receptor density and nano-organizational features on cancer cell surfaces, this technology enables accurate analysis of exosomes, offering insights for biomedical research and applications like liquid biopsy. These advancements deepen our understanding of cancer targets and nanoscale behaviour, facilitating early diagnosis and treatment evaluation for improved patient care. Thus, this review focuses on the development and challenges

of applying super-resolution imaging to cancer biology.

3. Super-resolution microimaging

Single-molecule localization techniques enable super-resolution imaging by distinguishing individual fluorophores in time rather than space. However, when multiple fluorophores are close, their PSFs overlap, hindering accurate localization. Methods like periodic photoconversion or scintillating moieties sequentially emit fluorescence for precise localization to address this. Despite variations in implementation, all SMLM techniques switch fluorophores between on and off states. Notable techniques include STORM and PALM, enhancing imaging resolution and empowering biology and materials science research.

3.1 Stochastic Optical Reconstruction Microscopy (STORM)

Stochastic Optical Reconstruction Microscopy (STORM) was developed by scientist Xiaowei Zhuang in 2006.[7] STORM utilizes the principle of single-molecule localization imaging by driving standard organic fluorescent molecules to a photoconverted dark state through a specialized buffer. The fluorophore cannot be excited in this dark state until it only returns to the ground state. Through the photoswitching properties of fluorescent dyes (e.g., Cy5 or Alexa647), each dye molecule can switch between the dark and fluorescent states under laser irradiation, emitting thousands of photons. By acquiring multiple frames, the PSF of each fluorescent molecule can be fitted by a Gaussian function to obtain the precise localization of each single molecule and generate super-resolution images.

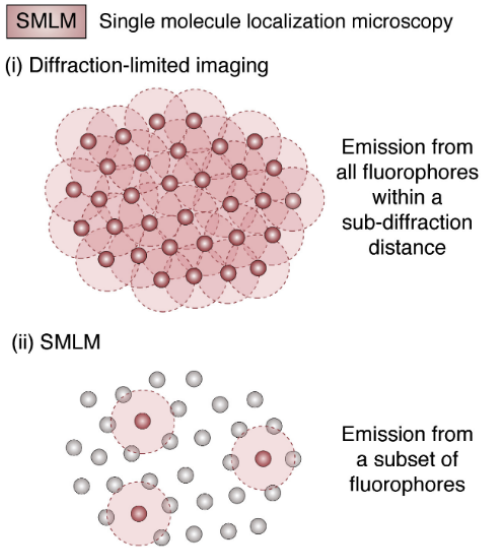

Fig. 2 Principle of stochastic optical reconstruction microscopy.

STORM requires repeated acquisition of multiple frame images until the desired number of localizations is achieved. The technique further simplifies sample labelling by displaying similar photoswitching properties, allowing the simultaneous use of several dyes with different excitation wavelengths. Optimizing buffer composition to maximize the entry rate into the dark state and minimize the escape rate from the dark state or photobleaching is often achieved by reducing buffers and reducing dissolved oxygen. Several studies have focused on optimal buffer compositions and provided detailed information.

Overall, STORM is based on random, massively parallel switching of individual fluorophores and requires prolonged acquisition under a wide-field TIRF microscope.[8] The technique reports higher resolution in optical imaging systems, capable of ~20 nm lateral resolution, and has demonstrated its utility in several biological problems, especially in imaging cancer biotargets.

3.2 Photoactivated localization microscopy (PALM)

The photoactivatable localization microscopy (PALM) technique was proposed in 2006 by Betzig et al.[9] PALM uses specific photoactivatable or photo-switchable fluorophores, usually genetically encoded or photosensitized fluorescent proteins. Common light-activated fluorophores are initially in an off state, where illumination at excitation wavelengths does not produce fluorescence. In contrast, light-switchable fluorophores typically photoconvert between shorter fluorescent species to longer wavelength forms, which can be unidirectional or reversible. A conformational change is induced by irradiating the fluorophore with an activation wavelength, which then allows the fluorophore to fluoresce at the excitation wavelength.

SMLM techniques have many applications in imaging biological targets in cancer. With their excellent spatial resolution, these techniques offer the possibility of selecting suitable organic dyes for labelling cancer cell-specific antibodies. Compared to conventional wide-field microscopy, this high-resolution imaging method allows us to clearly observe the details of nanoscale structures and cellular interactions, thus becoming a powerful tool for studying single-cell heterogeneity. Various findings have emphasized the importance of developing super-resolution techniques to detect and understand cancer at the single-cell level. Next, we will detail the application of SMLM technology to cancer targets to demonstrate its advancement and potential in cancer research.

3.3 Common Potential Biological Targets for Cancer

Early cancer detection reduces mortality rates, prompting significant investment in new detection technologies. Biomarkers, spanning nucleic acids, proteins, and more, offer valuable risk assessment, diagnosis, and treatment planning insights. With technological advancements, multidisciplinary diagnostic approaches are emerging, promising non-invasive tests for immediate and personalized cancer treatment. Biological targets like tumour antigens, circulating tumour cells, and microenvironmental factors are crucial. Through comprehensive analysis of these targets, accurate cancer assessment, treatment guidance, and monitoring can be achieved, ultimately enhancing patient outcomes and quality of life.

3.3.1 Cx43

Cx43, a connexin protein, is crucial in cancer progression and treatment. It forms transmembrane channels vital for chemotherapy pathways and facilitates intercellular communication, influencing tumorigenesis and interactions within the tumour microenvironment, including those involving non-tumour cells.

However, it is worth noting that no microscopy studies on the nanoscale have been performed to gain insight into the spatial arrangement of Cx43 in breast cancer cells.[10] More detailed studies are also needed on the effects of treatment outcomes (including ionizing radiation and antibody treatment) on the spatial arrangement of Cx43.[11] By fluorescent labelling with specific antibodies, the molecular location of Cx43 in the perinuclear cytoplasm and periplasmic membrane of cells in therapy-related applications can be determined using techniques such as SMLM, allowing in-depth study of Cx43 enrichment and molecular topological arrangement.

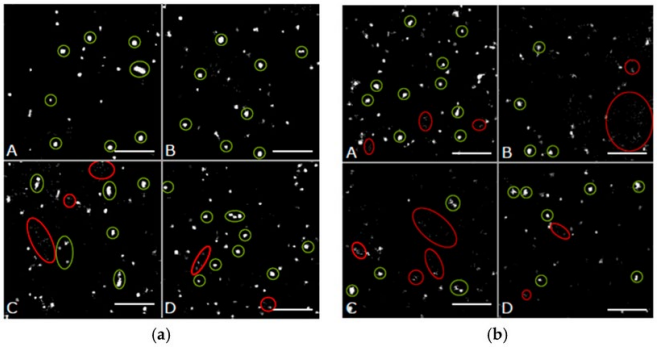

Fig. 3 Images of Cx43 molecules in HIMAEC by SMLM.

With the help of CLSM and super-resolution SMLM techniques, we could detect the effects of treatment on the spatial and functional arrangement of Cx43, thereby revealing the mechanisms of tumor response to it. Such studies are expected to provide important insights for a deeper understanding of the role of Cx43 in tumorigenesis and therapy.

3.3.2 HER2

Human Epidermal Growth Factor Receptor 2 (HER2), a member of the epidermal growth factor receptor family, plays a crucial role in cell growth and division under normal conditions. [12]However, overexpression or abnormal activation of HER2 can promote cancer development, particularly in breast cancer, where approximately 25% of cases exhibit high HER2 expression, known as HER2-positive breast cancer. This subtype tends to be more aggressive with a poorer prognosis.[13] Hence, HER2 serves as a vital biomarker for prognosis and treatment response in breast cancer. Accurate detection and quantification of HER2 are essential, typically conducted following ASCO/CAP guidelines using methods like fluorescence in situ hybridization (FISH) to assess HER2 gene amplification, aiding in determining HER2 status in breast cancer.

SMLM images of HER2 detected in breast cancer cell lines presented high-resolution intracellular molecular structures. These images demonstrate the distribution of the HER2 protein, exhibiting spatial resolution at the submicron level.[14] In addition, these images reveal the aggregation pattern of HER2 at the cell membrane and possible subcellular-level molecular arrangement patterns. Through SMLM technology, we could understand the distribution and localization of HER2 within breast cancer cells in greater detail, providing critical information for in-depth studies of the proteins role in tumour development and treatment response.

As a result, the SMLM technology excelled in detecting HER2 with superior spatial resolution and sensitivity, promising to predict response to therapy in clinical diagnostic tests. These findings establish nanotissues of HER2 as a potential marker of treatment-responsive disease and provide an essential reference for improving the accuracy of optimistic predictions for treatment-responsive breast cancer.

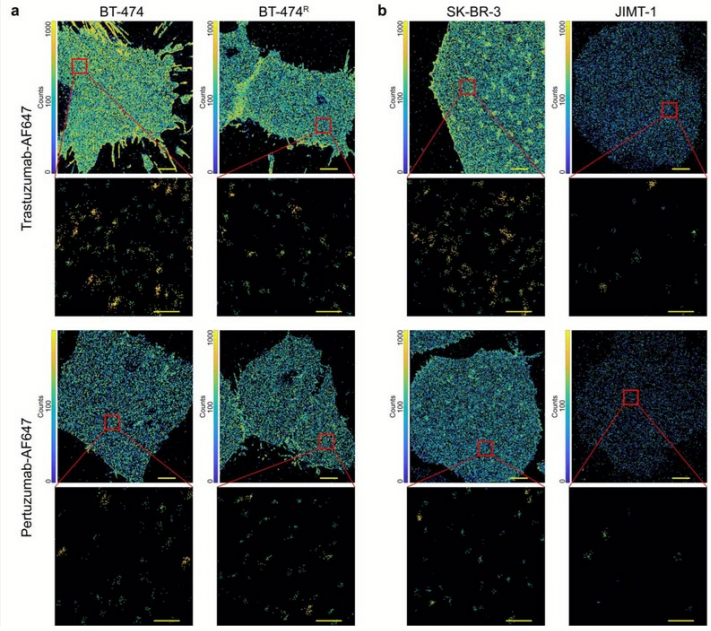

Fig. 4 SMLM images of HER2 detected in breast cancer cell lines.

3.3.3 EGFR

EGFR, or Epidermal Growth Factor Receptor, is a pivotal membrane receptor regulating cell growth, differentiation, and proliferation. As a tyrosine kinase receptor family member, EGFR modulates critical cellular signalling pathways. Its aberrant activation is strongly linked to various cancers like breast, lung, and colon cancers. [15]

Fig.5 SMLM imaging of the EGFR.

In cancer management, EGFR serves dual roles. Its over-expression or abnormal activation is a significant diagnostic marker, aiding tumour assessment and prognosis. Secondly, EGFR is a prime target for cancer therapy. Inhibitory drugs like gefitinib and erlotinib, which target EGFR activity, have gained widespread clinical use, particularly in non-small cell lung cancer treatment.

EGFR is pivotal in cancer development and a prime target in cancer therapy. [16] Super-resolution imaging of EGFR has advanced significantly, offering microscopic nanoscale assembly insights. Small-molecule inhibitor fluorescent probes enable detailed examination of EGFR, unlike bulky antibodies. Small-molecule inhibitors, like gefitinib, can penetrate cell membranes, binding to intracellular sites without compromising membrane integrity. It facilitates single-molecule study of drug-cell interactions, deepening our understanding of cancer therapy mechanisms.

3.3.4 PTK7

PTK7, a membrane receptor, is pivotal in cellular processes like polarity and motility, with high expression observed in primary adenocarcinomas. Due to its association with tumorigenesis and prognosis, it is a potential therapeutic target in non-small cell lung cancer. [17]Modulating PTK7 expression reduces cancer cell activity, promotes apoptosis, and hinders tumour growth, offering novel avenues for cancer treatment.

Moreover, PTK7s elevated expression is a vital marker in cancer screening, aiding tumour malignancy assessment and prognosis determination. Utilizing PTK7 levels assists in tailoring treatment plans effectively. Consequently, PTK7 holds promise for clinical use in cancer screening, potentially emerging as a diagnostic marker or therapeutic target, facilitating precise and early cancer diagnosis and treatment.

Super-resolution imaging (SMLM) has also been widely used to study intermolecular interactions in complex cellular environments. Chen et al. utilized aptamer probes and STORM technology to image PTK7 on MCF10A cell membranes. They investigated the effects of three cell membrane compositions (lipid rafts, actin cytoskeleton and carbohydrate chains) on PTK7 protein interactions. The results showed that all three cell membrane compositions affected PTK7 interactions on MCF10A membranes and that there were different degrees of effect between the basolateral and apical membranes. It suggests that super-resolution imaging is uniquely suited for observing the dynamic distribution and interactions of PTK7 on cell membranes, providing essential tools and insights for a deeper understanding of its functional mechanisms. [18]

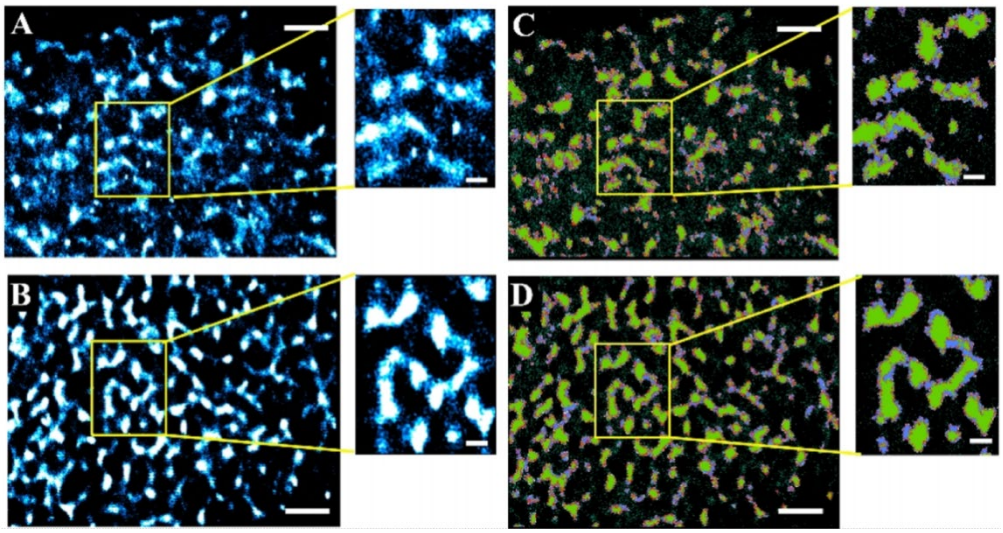

Fig. 6 Comparative map of PTK7 distribution on MCF10 parietal and basement membranes.

Despite its recent development, SMLM imaging technology, utilizing various probes, demonstrates significant potential. It categorizes cancer-related biological targets into proteins, DNA, and RNA, offering new avenues for studying complex cellular behaviours and nanostructures.

In cancer research, SMLM is crucial. It elucidates molecular mechanisms in cancer cells, aiding in understanding proliferation, metastasis, and drug resistance. Additionally, it enhances cancer diagnosis by providing precise molecular-level information and facilitating early diagnosis and classification. Moreover, SMLM assists in optimizing treatment plans and developing new therapeutic strategies by observing drug mechanisms in cells. Lastly, SMLM holds promise for assessing cancer prognosis by monitoring molecular characteristics and microenvironmental changes in cancer cells, aiding in predicting disease progression and patient prognosis.

In conclusion, SMLMs application in cancer research offers a valuable tool for scientific inquiry and provides an innovative and reliable approach for clinical diagnosis, treatment, and prognosis assessment.

4. Conclusion

This paper delves into single-molecule localization microscopy (SMLM) principles and their significance in cancer research. It comprehensively discusses super-resolution imaging techniques such as PALM and STORM, emphasizing their strengths and limitations. Furthermore, it explores cancer biotargets at the protein, RNA, and DNA levels, underscoring SMLMs role in deciphering nanostructure organization. Given the importance of understanding cancers molecular processes, SMLM emerges as a valuable tool for identifying molecular targets and potentially facilitating personalized cancer therapies through disease evolution tracking during treatment. The emergence of fluorescence nanography, a novel imaging technique, holds promise in revolutionizing biomedical research by visualizing biomolecules and their interactions at the nano- and single-molecule scale, offering new avenues for combating significant diseases like cancer.

However, there are notable practical challenges despite the promising potential of super-resolution imaging techniques like SMLM in unravelling cancer biology and aiding drug discovery. These include issues such as the photostability of fluorescent proteins, the rate of photoactivation, fluorescence intensity, potential artefacts in image reconstruction algorithms, and the time and cost associated with high-precision localization and optimizing microscope structures. Advancements in super-resolution optical imaging technology will continue to strive for breakthroughs in these areas, thereby advancing cancer research and treatment development.

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