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Reconstructing Super-Resolution Microscopy Images to Resolve T-Loop Structures of Telomeres

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ABSTRACT

The link between the macroscopic structure of telomeres and cellular health is an unanswered question in cellular research. An obstacle is the fact that conducting microscopic research on telomeres is challenging due to their small size limiting observations to the diffraction limit of light on standard optical microscopes. The novel techniques of super-resolution allow us to successfully image and accurately describe the macroscopic structure of telomeres. This takes steps towards uncovering more about the nature of telomeres and DNA.

GOAL

Establish an analytical workflow to visualize t-loops within human telomeres and improve current methods of telomere analysis using super-resolution methods of STORM and SRRF.

INTRODUCTION

Chromosomes contain most, if not all, the genetic information of living organisms. Degradation of this information has been linked to cancer, aging, and disease, making protection and preservation of this information an important goal. Telomeres, found at the end of chromosomes, have been suggested to protect DNA by forming a loop structure, coined as “t-loops”. This prevents ends from being identified as double-strand breaks and activating undesired DNA damage responses.

Human telomeres are short, ranging from 7 to 1 microns in length with the t-loop being only a small portion of that telomere. When conducting research on t-loops, light itself becomes the barrier when using a standard optical microscope as diffraction places a limit on resolution of around 200nm. This diffraction limit is a major obstacle when identifying the fine t-loop structures.

Super-resolution microscopy are newly developed methods of circumventing this diffraction limit. Stochastic Optical Reconstruction Microscopy (STORM) and Super- Resolution Radial Fluctuations (SRRF) both use the technique of capturing sequential images and reconstructing a super-resolved image, achieving up to an order of magnitude increase to 20nm of resolution.

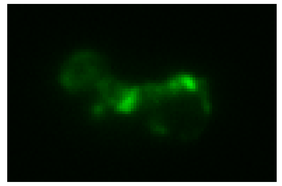
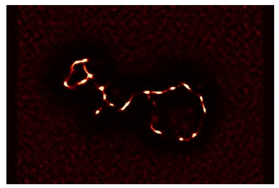
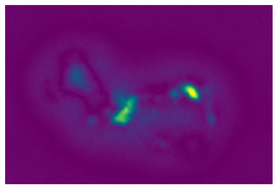
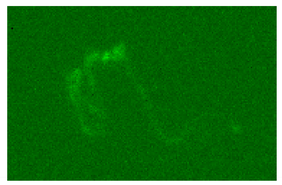
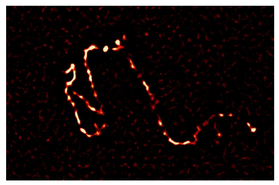
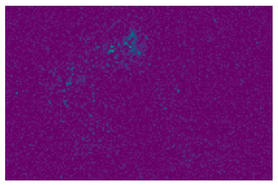
METHODS

1. Sample preparation
   - Samples are prepared by labeling telomeres with Alexa Fluor 647, a dye that is capable of photo-switching between dark and emissive states when combined with a STORM buffer, conjugated with peptide nucleic acid (PNA) probe to bind with telomeres.
   - Other biomolecules can be labeled as well, such as cell membrane with DAPI for multi-channel imaging.
   - Slides are then prepared with a chromatin spread for t-loops or a metaphase spread for general telomere analysis.
2. Imaging
   - STORM imaging was performed on the Quorom Diskovery Flex Multi-Modal Microscope with a 637 nm laser.
   - SRRF imaging was performed on the Nikon Ti Eclipse Wide-Field Microscope with a 630 nm laser.
   - Varied exposure times, frame numbers, and laser powers.
3. Reconstruction

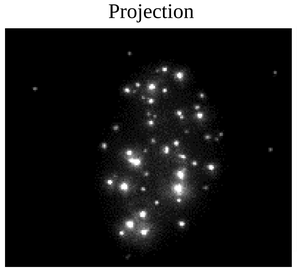
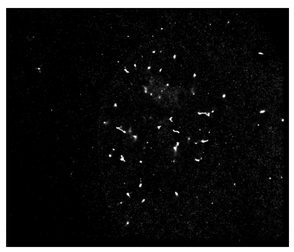
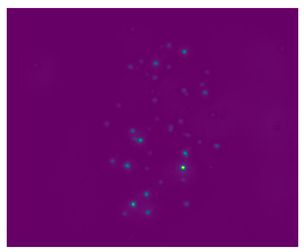
   Stochastic Optical Reconstruction Microscopy (STORM)
   - Utilizes photo-switching fluorophores that have a distinguishable dark and emissive state.
   - By only photoactivating a few random fluorophores per frame, this eliminates neighboring fluorophores from emitting photons at the same time which causes diffraction.
   - Every photoactivation detected on each frame is localized to determine a precise and accurate location of the fluorophore.
   - The localizations from each frame are compiled into a list of xy-coordinates and a single super-resolution image

   Super-Resolution Radial Fluctuations (SRRF)
   - Utilizes fluctuating pixel intensities in image sequences such as due to the random nature of photon emission and detection.
   - Finds sub-pixels that are consistently bright relative to its surroundings using a gradient map (radiality)
   - The gradient maps of each frame are autocorrelated to reconstruct a super-resolution image of the sub-pixels with high correlation.
4. Evaluating
   - Super-Resolution Quantitative Image Rating and Reporting of Error Location (SQUIRREL) quantitatively rates reconstructions by comparing pixel intensities of the reconstruction against the diffraction limited image.
   - Calculates the resolution-scaled Pearson coefficient (RSP), resolution-scaled error (RSE), and an error map.
   - Maximized RSP and minimized RSE and error map.

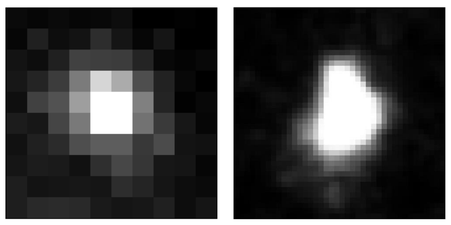
RESULTS

Wide-field Image	SRRF Reconstruction	SQUIRREL error map	Reconstruction Parameters
			Temporal Radiality Maximum Gradient Smoothing Brightness/Contrast Adjusted RSP = 0.410 RSE = 283.203
			Temporal Radiality Average Gradient Smoothing Gradient Weighting Brightness/Contrast Adjusted RSP = 0.482 RSE = 6.882

Images of spread immortalized human telomeres taken on Nikon Ti Eclipse Wide-Field Microscope (20ms exposure, 300 frames, 60x NA)

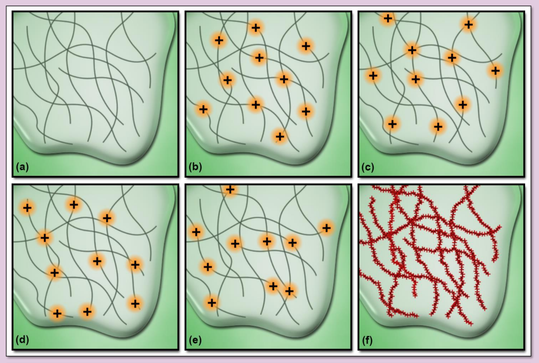
Confocal Maximum Intensity Projection	STORM Reconstruction	SQUIRREL error map	Reconstruction Parameters
			Wavelet Filter Local Maxima Integrated Gaussian Multi-Emitter Fitting Analysis RSP = 0.498 RSE = 252.162

Images of metaphase human telomeres taken on Quorom Diskovery Flex Multi-Modal Microscope (10ms exposure, 30 000 frames, 63x NA)



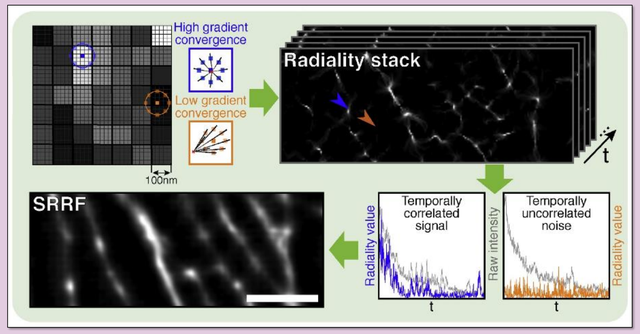
When analyzing individual telomeres in conventional image processing software, such as ImageJ, the standard confocal microscope image (left) renders a only a few pixels which limits information. Compared to the STORM reconstruction (right), more pixels can be used to describe the same object, thus providing more information.

Imaged cells are not to scale and have been magnified for this poster.



Reconstruction process of STORM

Image from Nikon MicroscopyU



Reconstruction process of SRRF

Image from Culley, S. et al.

CONCLUSIONS

- Images of chromatin spreads reconstructed with SRRF showed great success in resolving t-loops on immortalized human telomere.
- Preliminary trials of STORM within a metaphase spread showed a significant improvement in resolution.
- Using SQUIRREL to evaluate different reconstruction parameters, we found that different imaging conditions would require different parameters for the optimal reconstruction.
- The list of STORM localizations on an xy-plane were inputted into Ripley’s K function which showed clustering of localizations at distances within the average telomere radius.

Future Work

- Immortalized human telomere are still longer than regular human telomere, and evidence shows that telomeres shorten even further with age. With this workflow established for super-resolution imaging, more slides of chromatin spreads at various ages should be prepared to search for any links between the number of t-loops, telomere length, and cell age.
- To our knowledge, observing t-loops in old human telomere has yet to be accomplished, and the mechanism of t-loop formation has a multitude of unanswered questions. Finding a method of increasing t-loop count in chromosomes at all ages could assist in preventing DNA damage such as seen in aging and cancer.
- The improvement of resolution on metaphase spreads would be extremely useful in improving calculations of 2D area and 3D volume performed on telomeres by conventional image processing software, such as ImageJ.
- A workflow of cluster analysis on STORM localizations was not established. This is due to the stochastic nature of fluorophores that photobleach and then photoactivate again multiple times meaning there can be multiple localizations from a single fluorophore. This creates uncertainty that the clustering of localizations may not truly represent clustering of fluorophores, let alone the clustering of molecules.

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