CHROMAVISION

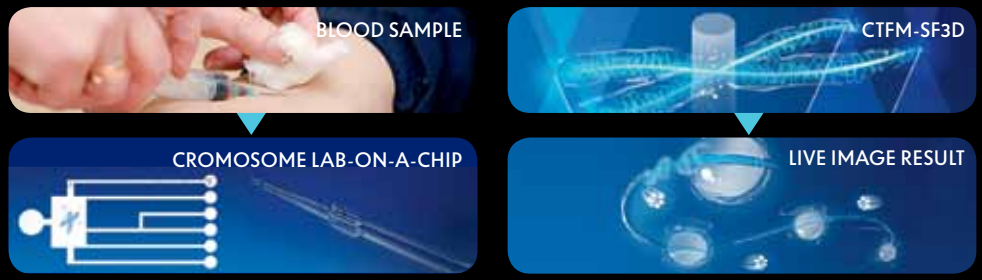
Pioneering chromosome imaging and manipulation platform

"This project has received funding from the *European Union's Horizon 2020 research and innovation programme* under grant agreement No 665233."

CHROMAVISION

CHROMAVISION aims to develop a pioneering chromosome imaging and manipulation platform that will fuel the next decades of structural chromosome research. Chromosomal abnormalities are characteristic of many disorders such as cancer, impaired fertility due to maternal aging, and neurological disorders such as fragile X syndrome. If humanity is to fully understand the wide range of diseases that are associated to errors in cell division, we must be able to further 'zoom in' on healthy and diseased chromosomes in all their complexity. The CHROMAVISION platform will allow molecular biologists to automatically isolate individual chromosomes from small tissue or cell samples and have these delivered to a super-resolution microscope. Chromosome isolation and delivery is achieved by an opto-fluidic chip that is able to trap, visualise and lyse individual cells and separate metaphase chromosomes from cell lysate. Single chromosomes can be "hand-selected" and brought into focus of the Super-Resolution Correlative Tweezers Fluorescence Microscope (CTFM-SR3D) that is developed in CHROMAVISION. This instrument will for the first time enable 3D, super-resolution, real-time metaphase chromosome observation and manipulation studies under near-physiological conditions. The technique will push the boundaries of what is currently possible in microfluidics and super-resolution microscopy and combine these into a single powerful approach for chromosome studies. Furthermore, the platform will be applied in CHROMAVISION to address key challenges in clinical and fundamental chromosome research, potentially resulting in breakthrough discoveries. Better imaging and understanding of the chromosomal mechanisms will contribute to our knowledge of the etiology of human diseases and aid drug discovery. The platform will also have large clinical value, allowing identification and monitoring of e.g. cancer heterogeneity.

CHROMAVISION flow-chart



The CHROMAVISION platform will allow molecular biologists to automatically isolate individual chromosomes from small tissue or cell samples and have these delivered to a super-resolution imaging system; the Super-Resolution Correlative Tweezers Fluorescence Microscope (CTFM-SR3D).
This instrument will for the first time enable 3D, super-resolution observation of metaphase chromosomes in real time, and permit manipulation of these chromosomes under near-physiological conditions. This technique will push the boundaries of what is currently possible in microfluidics and super-resolution microscopy, and will combine these into a single powerful approach for chromosome studies.

UNIVERSITY OF COPENHAGEN

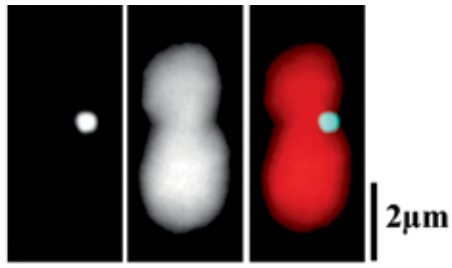
Department of Cellular and Molecular Medicine
Prof. Dr. Ian Hickson – Center for Chromosome Stability

Core business

The Hickson/Liu laboratory is based in the Center for Chromosome Stability (CCS) at the University of Copenhagen. The CCS was established in 2015 through a grant of 8.5 Mio Euro from the Danish National Research Foundation. The CCS's mission is to understand how cells minimize damage that can generate chromosomal instability. The main focus is on regions of eukaryotic genomes that are intrinsically unstable, including chromosomal fragile sites and repeated DNA sequences (e.g. telomeres). The long-term aim is to understand how genome instability triggers age-associated disorders in humans, including cancer, neurodegeneration and impaired fertility.

Research related to the project

The Hickson/Liu laboratory will provide expertise in molecular/cell biology within the Chromavision consortium. We aim to develop the technology to attach molecular 'handles' to human chromosomes, permitting controlled manipulation of chromosome structure in-vitro. We are experimenting with both in-cell and in-vitro attachment of handles to defined regions of the chromosomes – principally the centromeres (where the mitotic spindle attaches to chromosomes) and telomeres (the ends of chromosome). These handles will be designed to permit attachment of magnetic spheres or other microspheres for subsequent controlled manipulation via the use of 'tweezers'. In parallel, we are genetically manipulating human cells to make alterations that mimic those found in certain human diseases; in particular cancer and neurological conditions. This will allow us to define in detail the effects that disease mutations have on chromosome stability and function. Ultimately, we hope to use a cell-free system to reconstitute in-vitro key events that occur during the mitotic phase of the human cell cycle, such as the disjunction of the two sister chromatids that comprise a human chromosome.



Development of a system to attach a molecular 'handle' to the centromere of a human chromosome. A DAPI-stained human chromosome (red) that has a micro-bead attached specifically at the centromere – the constriction point of the chromosome where the mitotic spindle attaches.

DENMARK TECHNICAL UNIVERSITY

Prof. Dr. Anders Kristensen – Optofluidics

Core business

The research includes photonic crystal sensor surfaces for refractometric imaging, opto-thermal actuation and laser manipulation, nanoplasmonics for integrated delivery of light at the nanoscale, and plasmonic color metasurfaces for decoration and laser nano-lithography. This research is supported by polymer micro-fabrication technology research and development .

Research related to the project

In Chromavision, DTU Nanotech has focus on developing production grade, injection moulded plastic chips for the Chromavision platform. This includes design and validation of cell traps, on-chip lysing, and chromosome extraction as well as integrated optics for capturing and delivery of individual chromosomes.

Chromosome Chip DTU is working on

VRIJE UNIVERSITEIT AMSTERDAM

Department of Physics and Astronomy (coordinator of the project)
Prof. Dr. G.J.L. (Gijs) Wuite – Physics of Living Systems

Core business

My group is part of the Physics of Living Systems Section in the faculty of Sciences at the Vrije Universiteit Amsterdam. The research in the group focuses on exploring DNA-proteins interactions and biophysical/biomechanical properties of viral capsids & cells. The aim is to work with increasingly more complex assemblies of proteins in order to investigate the emergent properties from these systems. This approach bridges experimental systems biology and single-molecule manipulation techniques. We use a variety of techniques such as optical tweezers, AFM and single-molecule fluorescence as well as combinations of these techniques. The data obtained are related to biochemical studies and used for theoretical modeling.

Research related to the project

1. 3D STED imaging of stationary metaphase chromosomes

The focus here is to bring imaging of metaphase chromosomes to a new level of resolution and control. The condensed chromosomes will be flown in our microfluidic chamber and captured out of solution using the specific chromosome-microsphere handles. We will apply different imaging approaches, starting with confocal fluorescence microscope and 2D-STED approaches while holding a chromosome with two optically trapped microspheres, and ultimately aiming for 3D-STED imaging of chromosomes held with four microspheres. We envision that this super-resolution imaging of different kinds of chromosomes will result in deep insights in chromosome structure.

2. Determine the mechanical properties of healthy and 'diseased' chromosomes

In this project we will enhance our understanding of the mechanical and structural properties of chromosomes by combining micro-mechanical manipulation with super-resolution imaging. The experiments will yield information in the differences between the structural and mechanical properties of healthy and diseased chromosomes. Our aim is to reveal correlations between protein structures and the mechanical response of chromosomes to elucidate the nature/origin of chromosome architecture as well as defects.

3. Follow, in real time and with molecular resolution, the segregation of metaphase chromosomes triggered by the introduction of separase and Topoisomerase II

Finally, we are planning to trigger and follow the dynamics of a segregating chromosome. The result of this task will be highly detailed spatial and temporal observations of the events taking place during the first steps of chromosome segregation. Such data is far beyond the current state of the art and would allow a biological understanding of segregation currently unthinkable.

LUMICKS

Capture Molecular Interactions
Olivier Heyning

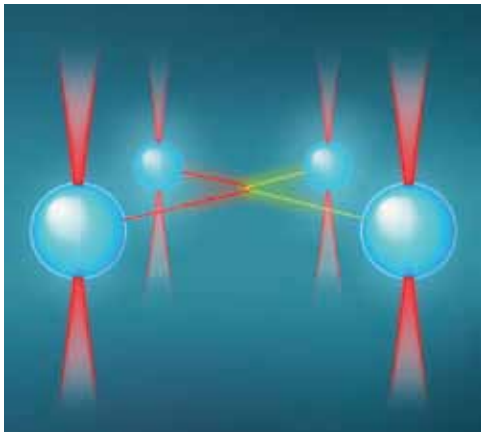
Core business

LUMICKS is a spin-off company from the Vrije Universiteit Amsterdam, bringing to market the revolutionary C-Trap Correlative Tweezers (CTFM) and Acoustic Force Spectroscope (AFS). The instruments enable live visualization of DNA-protein interactions at single molecule level in real time. LUMICKS provides ready-to use single-molecule instrumentation, give understandings to the complex biological processes at single molecule level, which is key for prevention and cure of Cancer and other diseases.

The C-Trap combines optical tweezers, confocal microscopy or STED nanoscopy and an advanced microfluidics system in a truly integrated and correlated way. The C-Trap is capable of live, simultaneous and correlative visualization and manipulation of molecular interactions with sub-picoNewton force resolution and sub-nm localization. Image: © LUMICKS

Research related to the project

LUMICKS incorporate the technical developments of CHROMAVISION in their new generation C-Trap that can be widely implemented. By doing so, LUMICKS provides a unique single molecule instrument that enables researchers to study complex multi-step biological processes on the molecular level with unprecedented clarity. The new generation C-Trap will be capable of simultaneously visualizing and manipulating single molecules - up to full chromosomes - with sub pN force resolution and single photon sensitivity. This is enabled by the CTFM's four continuous optical traps with a wide ~100 um Field of Operation and large range in trap stifness, along with fast 3D STED scanning. LUMICKS is involved and/or leads the R&D projects that are related to progressing the CTFM- technology, such as: wider Field of View for STED and fluorescence, wider Field of Operation for the four continuous optical traps and 3D STED. In addition LUMICKS develops a solution to integrate the necessary microfluidics into the CTFM platform and the necessary software.



Artist impression of four optical traps holding chromosomes in metaphase image: © LUMICKS

UNIVERSITY COLLEGE LONDON

Prof. Dr. Charles Swanton

Core business

The Swanton laboratory focuses on mechanisms generating cancer genetic diversity and its consequences on clinical outcome. Our group and others, through next generation sequencing studies, have demonstrated that the principles of Darwinian evolution apply to the growth and adaptation of human tumours.

Our group has demonstrated that intratumour heterogeneity, through tumour sampling bias, impacts upon our ability to successfully qualify cancer biomarkers for clinical use. We have also found evidence for extensive parallel evolution in human tumours, with multiple spatially separated subclones acquiring distinct mutations in the same gene, protein complex or signal transduction pathway, suggesting profound constraints to tumour evolution that might be exploited for therapeutic benefit.

CHROMAVISION

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