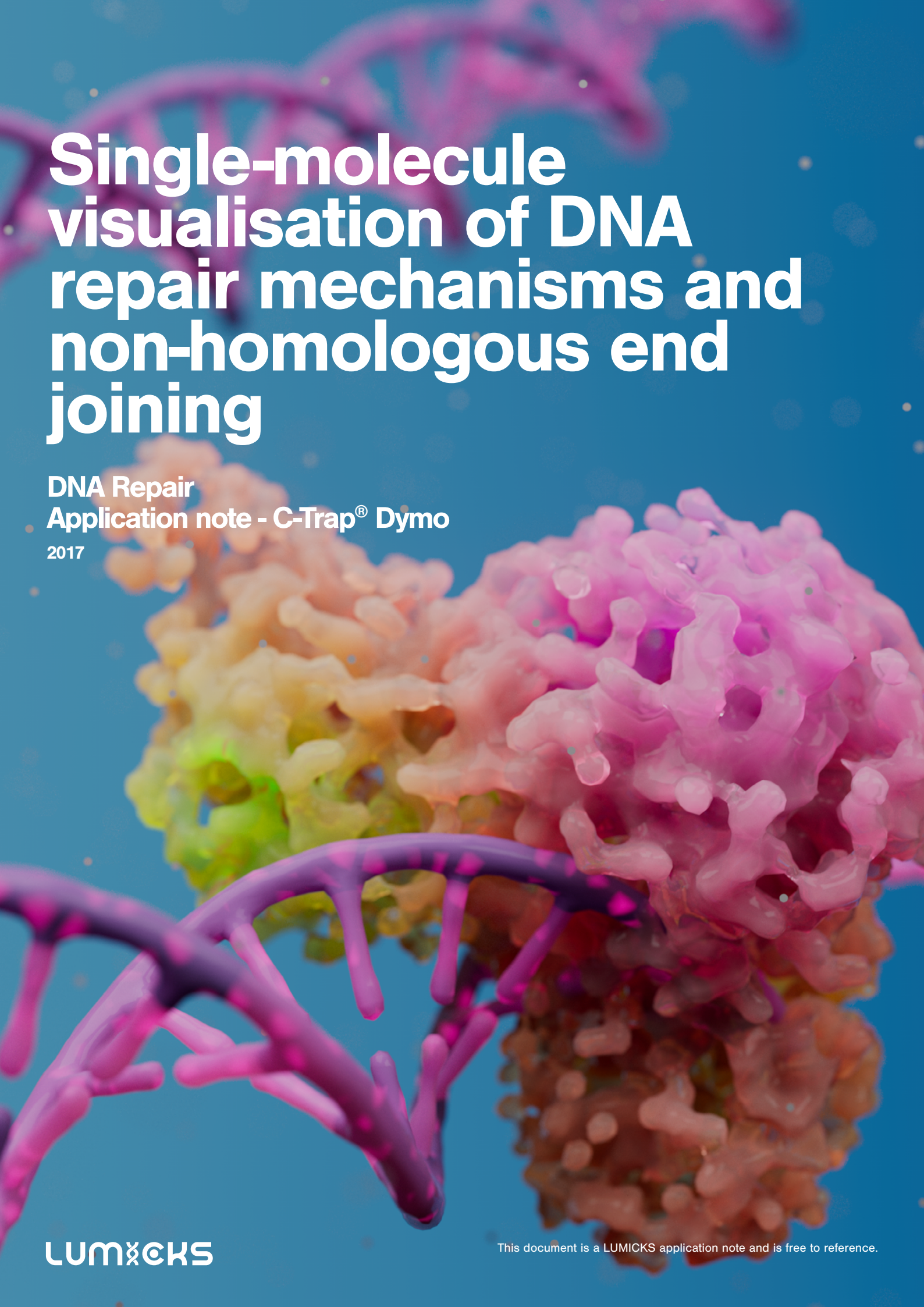
Single-molecule visualisation of DNA repair mechanisms and non-homologous end joining

DNA Repair
Application note - C-Trap® Dymo

2017



LUMICKS

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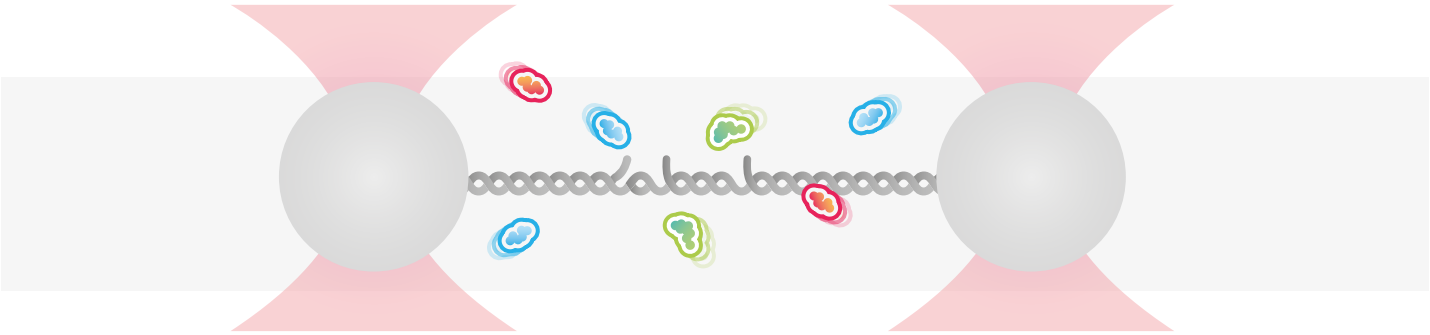


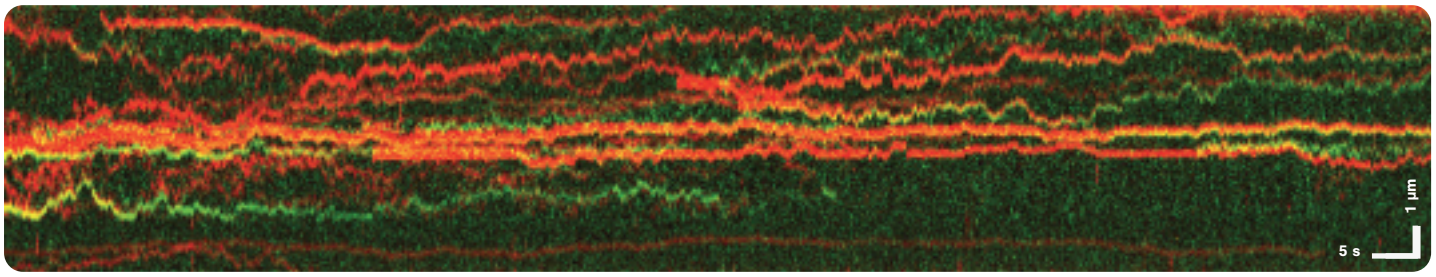
1 A schematic representation of DNA repair using an optical trap set-up.

Study Molecular Mechanisms Involved in DNA Repair

DNA repair, the collection of highly-regulated mechanisms by which a cell identifies and repairs DNA damage, remains one of the most essential processes of human life. Without DNA repair mechanisms cells lose the ability to transcribe important regions of their genome, resulting in harmful mutations, which could eventually jeopardize cellular wellbeing.

Sources of DNA damage include double-stranded breaks and DNA intra- and interstrand crosslinks, which can ultimately become malignant tumours, leading to cancer. To study DNA repair, single-molecule studies have proven to greatly enhance understanding at the molecular level. However, it is often challenging to obtain adequate sensitivity and resolution, as well as biological conditions mimicking *in vivo* environments. LUMICKS' C-Trap® Dymo allows for real-time visualization of the interaction between DNA and DNA repair proteins, under biologically relevant conditions with high spatial and temporal resolution.

The illustration above shows a DNA molecule tethered between two optically trapped beads and multiple DNA repair proteins interacting with the DNA. The position of the fluorescently labeled proteins is visualized over time by using a multi-color confocal beam, thus unveiling the position, diffusion and (un)binding events of the proteins along the DNA. Simultaneous force and extension measurements allow correlating the protein activity and binding kinetics with the mechanical properties of the DNA-protein complex.



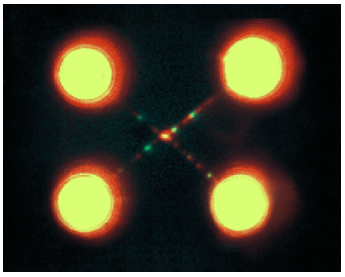
2 A kymograph. The X axis corresponds to time, while the Y axis corresponds to the positioning of the bound protein along the DNA, giving real-time insights into the DNA-Protein interactions of DNA repair.

With real-time, high resolution observation of DNA-protein interactions involved in DNA repair, the molecular activities and kinetics of this complex can be observed and studied. In order to do this using the C-Trap, DNA is tethered between two beads, as multiple fluorescently labelled repair proteins are interacting with the DNA molecule. Through multicolour confocal excitation and detection, the diffusion, movement and (un)binding events of the repair proteins can be visualized and studied.

The kymograph in **Figure 2** shows the binding position of XRCC4 (green, 9% of the total number of events) and XLF (red, 62% of the total number of events), two DNA repair proteins that are involved in non-homologous end joining (NHEJ) repair pathway and can form XRCC4-XLF complexes (yellow, 29% of the total number of events).

XRCC4 and XLF also play a role in DNA bridging. This can uniquely be studied with the C-Trap by adding two additional optical traps (Q-Trap®), allowing for an additional DNA molecule to be manipulated. In **Figure 3** two molecules were incubated with 200 nM of XLF and 200 nM of XRCC4 in a crossed conformation and bridge formation was subsequently visualized. We can observe that a bridge composed by the protein tandem is indeed present between the two DNA molecules. It is then possible to manipulate the beads with force to further validate bridge stability and to study the behaviour of the DNA-repair proteins under tension. For example, by exerting high force (>100pN) on the lower right bead and the top right bead (originally tethering different DNA molecules), the DNA tethers detach from these two beads. As a result, XLF-XRCC4 DNA repair protein complex maintain a bridge between the left bead, one of the initial steps during NHEJ to repair two broken DNA fragments. By then exerting tension on the new DNA fragment, the strength of the bridges can be determined. A significant fraction of the rupture events was observed at tensions up to 250 pN, demonstrating the high stability and strength of the protein bridges. The Q-Trap is the first technology in the world that allows such experiments to be performed.

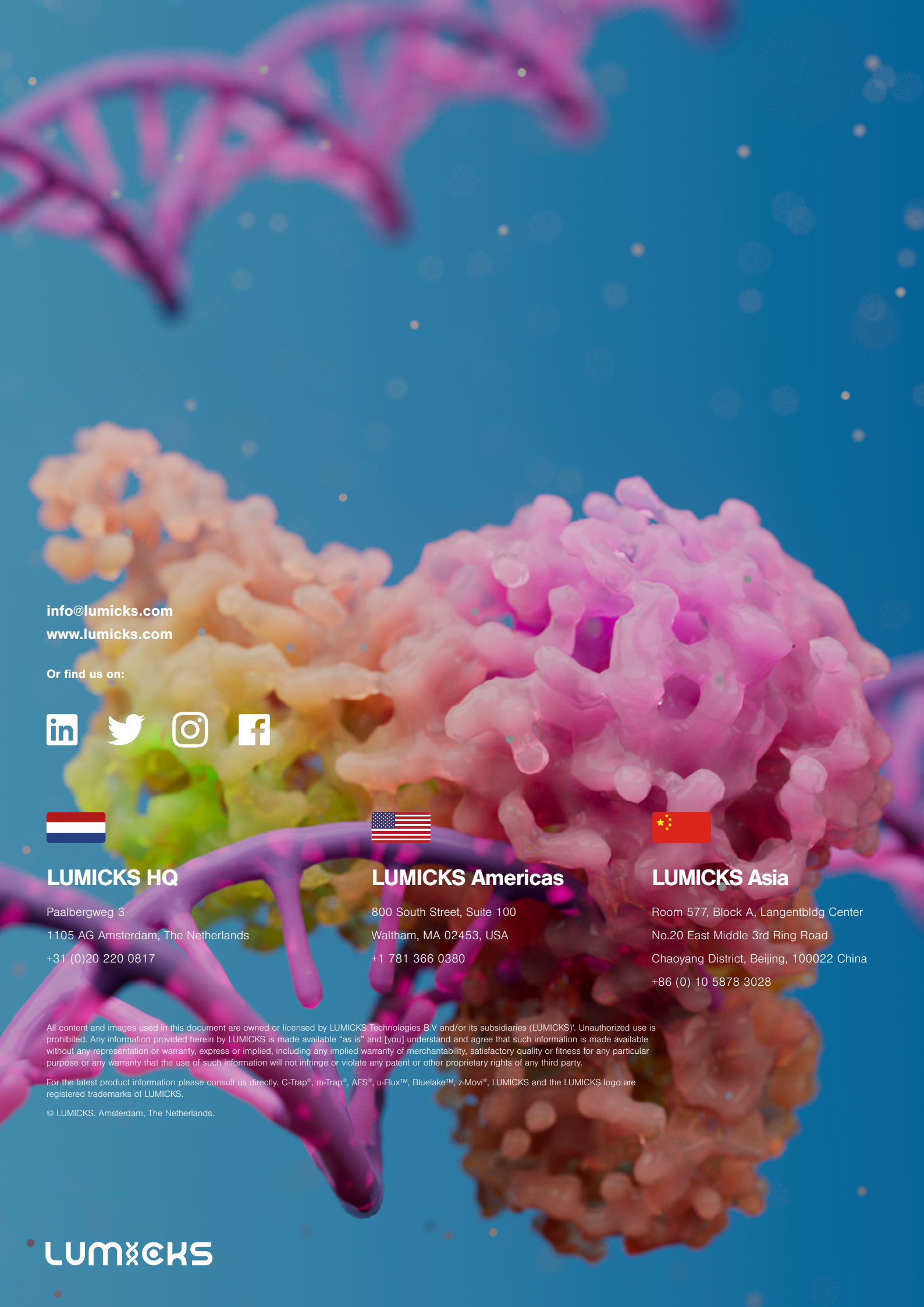
Additionally, because DNA repair often occurs in highly crowded environments, STED nanoscopy can also become an asset to distinguish between individual labelled proteins and filaments. This is done with a 745-nm 1D STED beam. More information about STED nanoscopy can be found on the LUMICKS website.



3 XRCC4 and XLF bridging two double-stranded DNA molecules in a cross conformation visualized by dual-colour confocal microscopy.

Read more Brouwer et al. "Sliding sleeves of XRCC4–XLF bridge DNA and connect fragments of broken DNA" Nature (2016)

Data courtesy of Prof. Dr. Gijs Wuite and Prof. Dr. Erwin Peterman at the Vrije Universiteit Amsterdam.



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