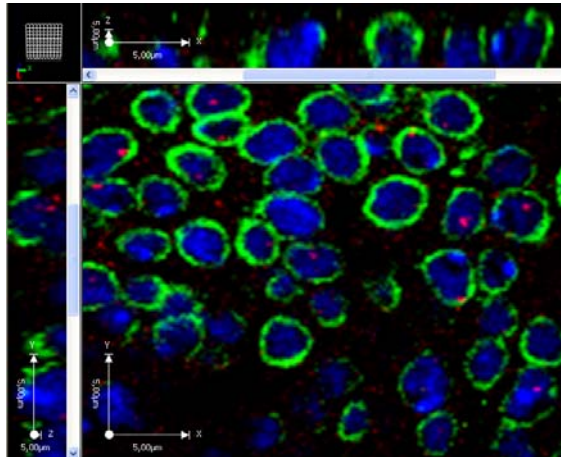




ImmunoFISH analysis in *Drosophila* Eye Disc



Volocity Image View in XY, YZ and XZ of a Z-stack of eye disc nuclei in presence of the Idefix retrovirus

Blue: DNA
Green: nuclei membrane
Red: DNA probe (marking the region of interest)

Position in the nucleus of each internalized DNA probe was calculated in 3D using **Volocity Classification**

Dr Emilie Brasset is part of a team headed by Dr Chantal Vaury at the Faculty of Medicine in Clermont-Ferrand, which studies genetic instabilities and host control in *Drosophila melanogaster*. The group looks at tiny DNA sequences called transposable elements which are able to move from one chromosomal site to another. Once inserted, a copy remains in the DNA and is transmitted from generation to generation, which can lead to mutagenic effects in a whole population. The team is particularly interested in two retroviruses named ZAM and Idefix and they aim to understand their influence on *Drosophila* genomic regulation.

The Idefix retrovirus causes mutagenic effects in the host genome by establishing a dialogue with genes surrounding its insertion site. Emilie focuses her research on understanding the role of a specific functional domain of Idefix in regulating the *Drosophila* genome subnuclear organization.

To achieve this she uses the Immuno Fluorescence In Situ Hybridization (ImmunoFISH) technique to accurately pinpoint a DNA sequence which is thought to be regulated by Idefix in the *Drosophila* eye disc chromosomes.

DNA probes binding to each extremity of the sequence of interest were tagged with digoxigenine and recognised by anti-Dig antibodies labeled with Rhodamine. Nuclei membranes were stained with anti-laminine antibodies recognised by Alexa 488 coupled antibodies and the chromosomes were stained with DAPI. Z stacks of the eye disc nuclei were acquired with 0.4µm z-step on a 63x objective of a LSCM. The data were collected in both the presence and absence of the retroviral transposon Idefix.

The Z stacks were imported into **Volocity** for analysis of the position of the sequence of interest. **Volocity Classification** allowed Emilie to automatically calculate the nuclear position of the internalized probe, and therefore to identify the exact position in 3D of the DNA sequence of interest in each nucleus and in both experiments. **Volocity Visualization** was also used as a powerful tool for fast and easy visualization and exploration of the acquired data in 3D.

The results obtained with **Volocity Classification** successfully showed that in presence of the retrovirus Idefix the position of the sequence of interest was modified. This suggests that the sequence that is being studied could play a role in marking a specific region in the *Drosophila* eye disc nucleus.