High-resolution 3D imaging of whole zebrafish ovary

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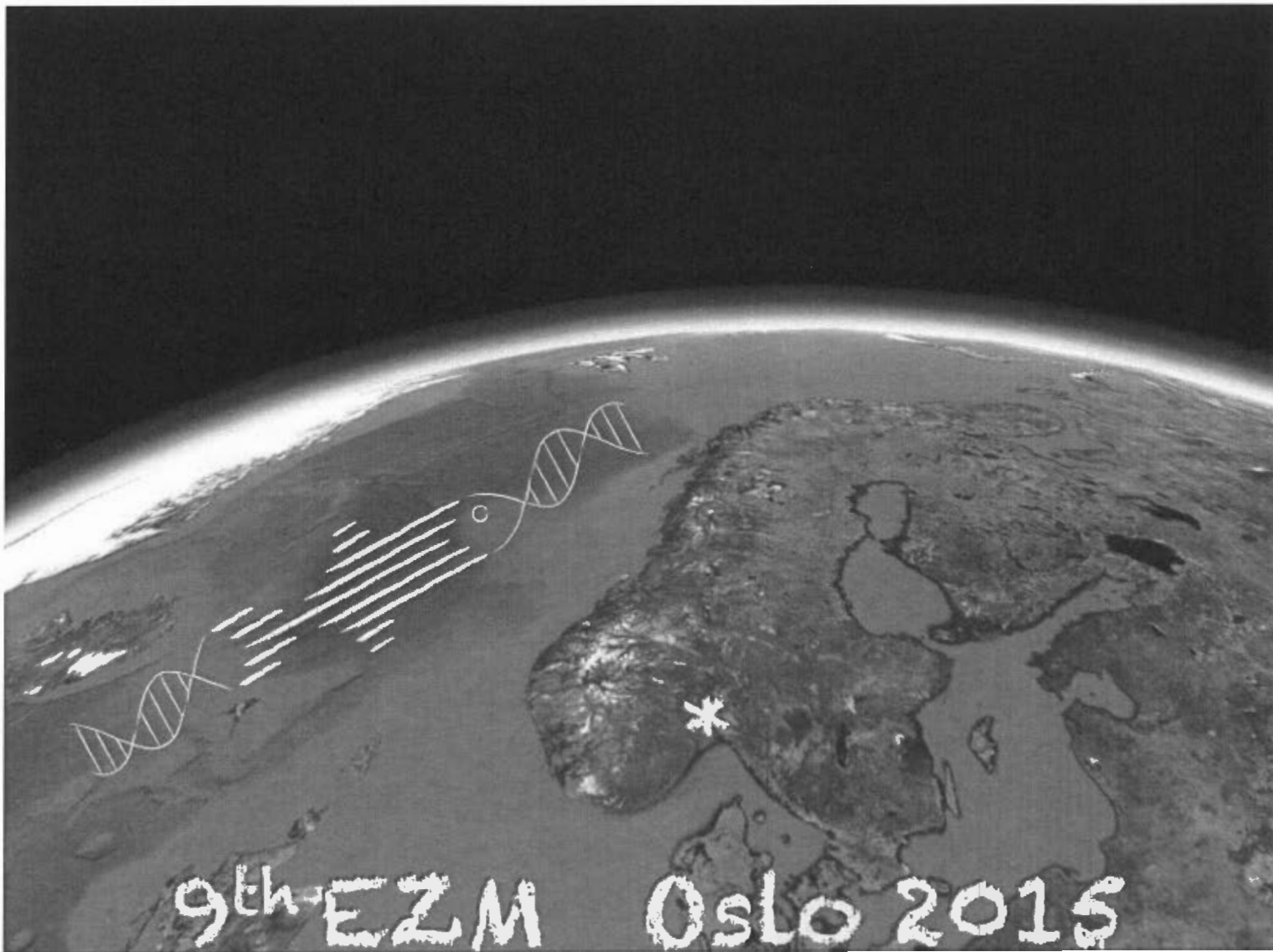

[P1-089] High-resolution 3D Imaging Of Whole Zebrafish Ovary.

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Transgenic animals expressing fluorescent proteins are important tools to characterize fish reproduction biology and pathologies. Traditionally, phenotypic analyses of ovaries are conducted on mechanical tissue sections, which do not give access to three-dimensional information. Three-dimensional imaging of biological tissues is limited due to light absorption and scattering by tissue component. Several techniques that render tissues permissive to light have been recently described. Here, we tested several clearing protocols (CLARITY, CUBIC...) on zebrafish ovaries. We established that CLARITY is the best-suited method for clearing ovary since it allows two-photon imaging until 300µm, compared to 40µm without treatment. Endogenous GFP fluorescence is conserved although the signal is weakened, and immunohistochemistry is possible. Furthermore, to achieve whole-tissue high-resolution imaging we combined the two-photon imaging of cleared tissue with automatic serial sectioning (VibMicTM, Leica). Mechanical sectioning and successive block-face imaging allowed us to go beyond the existing limits of conventional microscope objectives and to image the whole organ. We established a pipeline for 3D imaging of whole zebrafish ovary at cellular resolution within one month. Large sample dataset generated can thus be used to perform 3D morphological analyses and cellular quantifications at the whole organ level.