Equipment for the characterisation of plankton, from pico- to micro-, at community, population and individual levels.

FC, Flow Cytometry; IFC, Imaging Flow Cytometry; IF, Imaging-in-Flow; OM, optical microscopy; CM, confocal microscopy. The Table shows the type of equipment, size-range of the analysed particles (equivalent spherical diameter -ESD), and the objectives and tasks described in the Technical Annex of the BIOTOX project.

Equipment	Size-range (ESD)	Objectives
FC SH800 (Sony)	1 – 100 µm	• Physical sort of cells based on size and fluorescent properties in order to couple this data with OM and CF
IFC (Lab) (Amnis Flowsight) Laboratory	1 – 60 µm	• Estimation of *Alexandrium minutum* mitotic and meiotic rates based on DNA content distribution and morphological traits.
IFC (In situ) Compact, robust IFC for ex situ on board and in situ water column operations (in situ-IFC)	1 µm – 1 mm	• Phytoplankton community structure (assemblages) • Population dynamics of functional groups (guilds) and target species (Alexandrium spp. and other HAB species) • Eco-physiological traits (e.g. size and morphometric features, physiological state) of target species from their optical properties and image analysis of the acquired images
IF (Lab) (FlowCam)	5 – 300 µm	• Plankton community (both autotrophic and heterotrophic organisms) and non-living particles (e.g. detritus, faecal pellets) • Eco-physiological traits (size and morphometric features) from image analysis of acquired images

OM and CM (Leica)	Not limited	• Calibrate parasite versus host infected populations based on higher magnification analyses than that of IFC equipments (20x). • Characterize cell heterogeneity during the process of infection at higher magnification than that of IFC equipments (20x)

Technical description of the set of FC, IFC and Microscopy equipments to be used in BIOTOX

SH800 Sony (sorter) (FC). Conventional flow cytometer and automatic sorter equipped with one excitation laser (488 nm), side scatter, back scatter and 6 fluorescent parameters. This equipment allows -with the help of an easy and fast automatic calibration based on commercial chips, the physical sort of particles based on fluorescent or size properties and 7 levels of purity, depending on the needs of the application in use. For the study of intracellular infections, the possibility of sorting different populations based on DNA staining (parasite population, low levels of infected host, high level of infected host, etc.) will allow both 1) the interpretation of IFC data, 2) the analysis of infection traits under higher magnification.

Amnis Flowsight (IFC). Two excitation lasers, blue (488 nm) and violet (405 nm). Twelve standard detection channels simultaneously produce brightfield, darkfield (SSC), and up to 10 channels of fluorescence imagery of every cell (435-800 nm). It operates like a conventional flow cytometer, but also provides imagery of every cell (20x magnification), with the possibility of implement population statistical analyses based on more than 30 different morphological parameters, including morphological classification of subpopulations (for example, to distinguish between healthy and infected cells based on the morphological effects of infection) and the possibility of building classifiers using correlation of stained populations with morphological characters (for example, DNA stained cells versus morphological evidences of infection, in order to determine if some cell cycle/life cycle stages are more prone to be infected than others). This equipment also allows multiplexing (several staining protocols). See in the revision of Haridas et al. 2017 on the advantages of using IFC for the study of parasite infections (intracellular pathogen research).

IFC (In situ) (Anuncio de licitación: Suministro, instalación y puesta en funcionamiento de un citómetro-microscopio de flujo para análisis automatizado de plancton en laboratorio e in situ en la columna de agua. Expediente: CN063/21 -BOE Nº 280, Pag. 67899-67901, Martes 23 de noviembre de 2021)**.** Two excitation lasers, blue (488 nm) and green (552); two detectors of light dispersion, forward and sideward scatter (FWS and SWS respectively); and three fluorescent channels: yellow 550-600 nm (phycoerythrin), orange 600-650 nm (Chl-b and phycocyanin) and red >650 nm (Chl-a and Chl-b). Registration of optical properties of the particles, included pulse shape intensity of each emitted signal, which depend on the shape, internal structure (i.e. location of chloroplasts) and physiological state of the algal cells. Discriminant Analysis of Multi-Aspect CYtometry data (DAMACY) (Tinnevelt et al. 2017) applied to registered optical properties to describe subtle phenotypic changes within the phytoplankton population which, for instances, may indicate a minor toxicological change and or/infection from parasites. This will result in a fingerprint of every measurement, which can then be used to predict the interacting abiotic and biotic variables. Size of the

analysed particles range from submicron to millimetres (ca. 1 µm to 1 mm ESD) given the design of the fluidic system and scanning signal format. The collection of images allows particle identification and image analysis for the extraction of morphometric features. This equipment will be used in the laboratory (fresh and stained particles), on the observational studies during the seasonal cycle and bloom development (both ex situ on board and in situ immersed in the water column), and on autonomous mode for high frequency sampling during the mesocosm experiments.

FlowCam (B2 series) (IF). In-flow acquisition of images of the living and non-living particles present in the sample. Size of the analysed particles range from 5 to 300 µm. To be used in the laboratory (fresh samples).

LEICA SP8 (CM). Confocal microscope equipped with 3 laser lines (405 nm, 488 nm and 552 nm), bright field (PMT trans) in the transmitted light, 4 objectives at magnifications 20x, 40x, 63x and 100x and a super-resolution module (LIGHTNING).