

Generation of spheroids of cancer cells for screening of anti-tumour activity of cyanobacterial extracts and isolated compounds

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Introduction

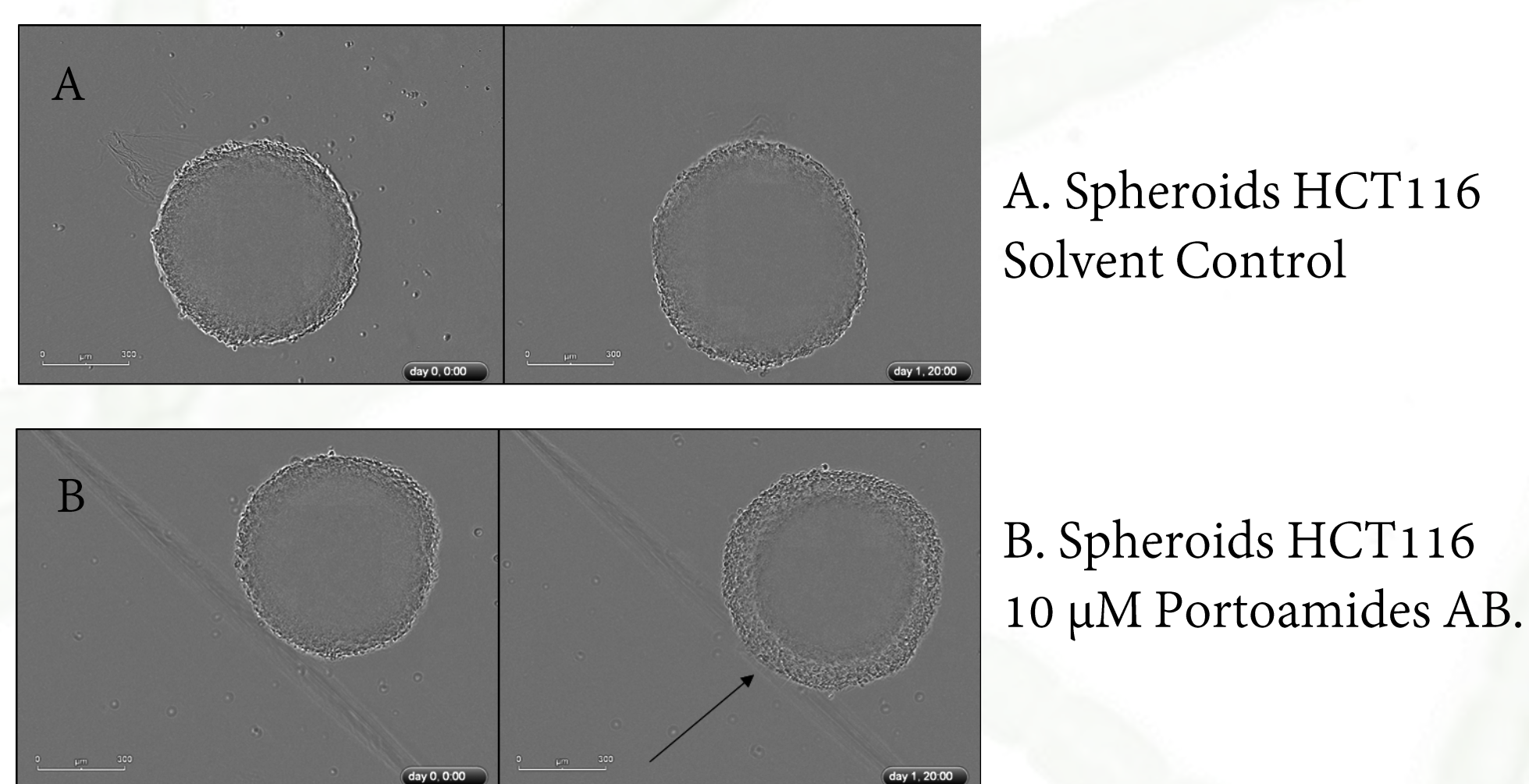
Cyanobacteria have distinctive characteristics namely the production of secondary metabolites which are seen as having a variety biotechnological applications, such as **anticancer**, which enhances their **potential as a source for new drugs**.

The use of **3D cell culture** for modelling cancer research is gaining interest.

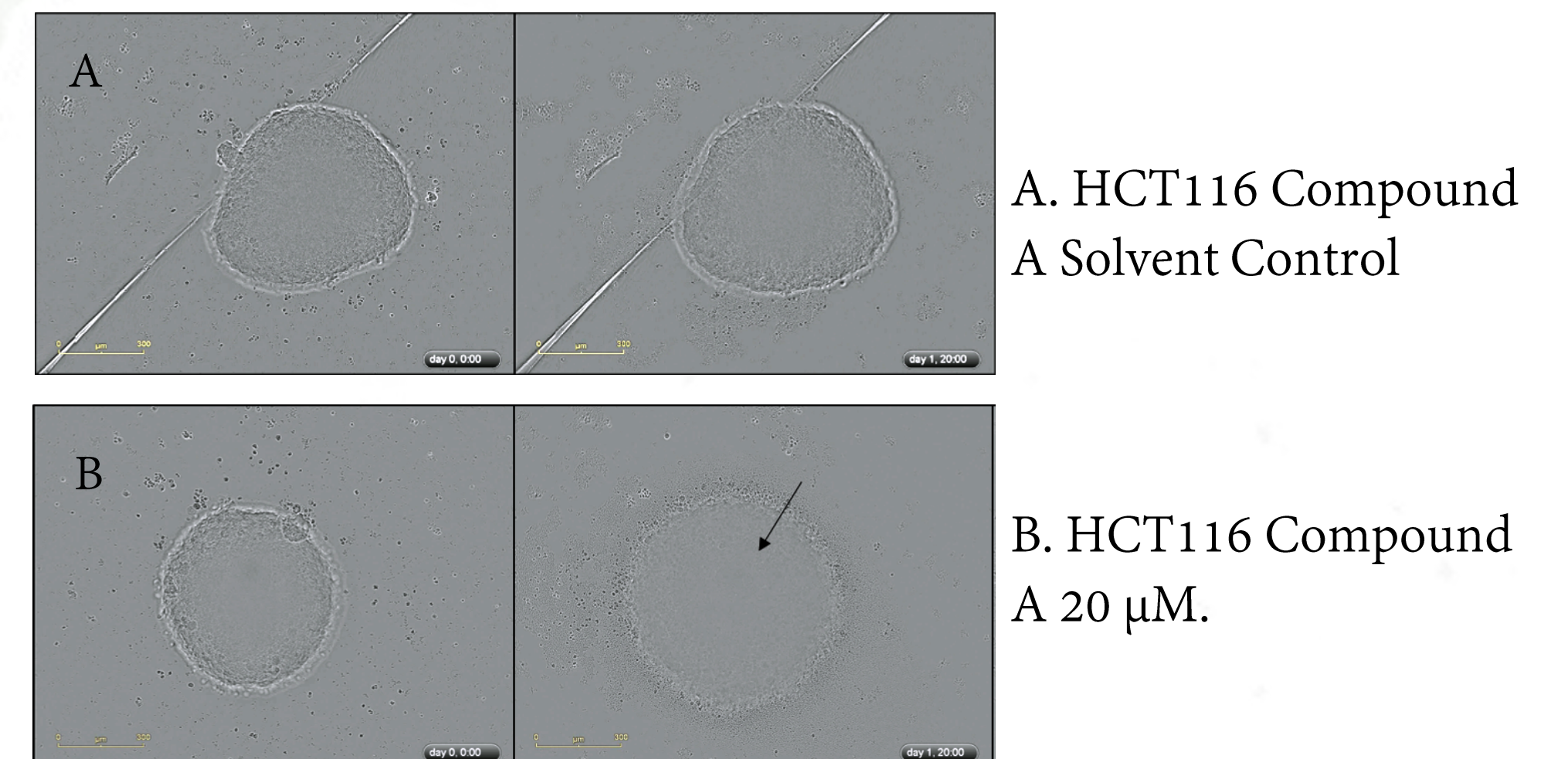
In the presented work, **3 compounds isolated from cyanobacteria** and **92 chromatographic fractions** obtained from crude organic extracts of 28 cyanobacterial strains from the Portuguese coast were tested on spheroids generated from the human cancer cell lines HCT11, HT-29 and A549.

Results

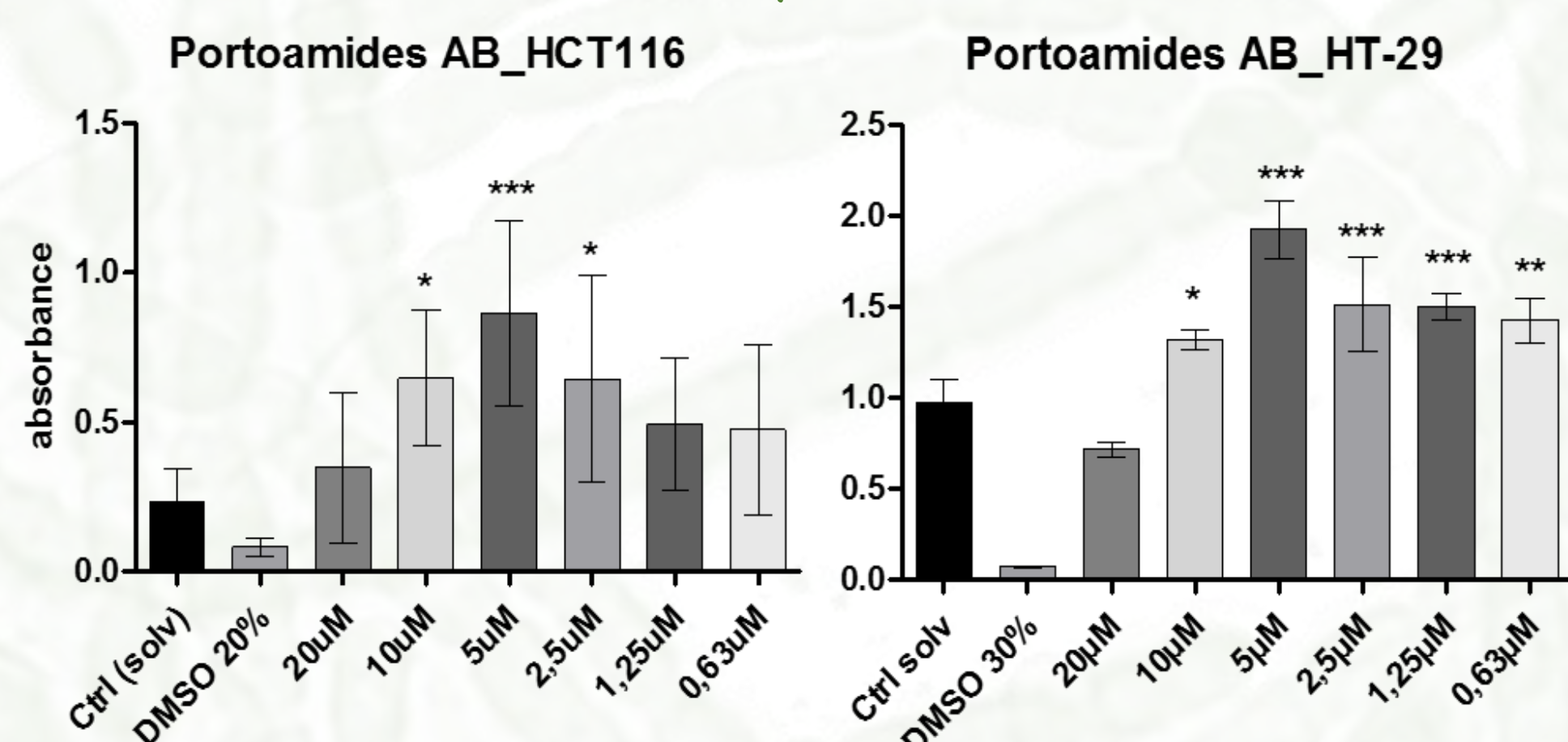
Portoamides AB



Compound A

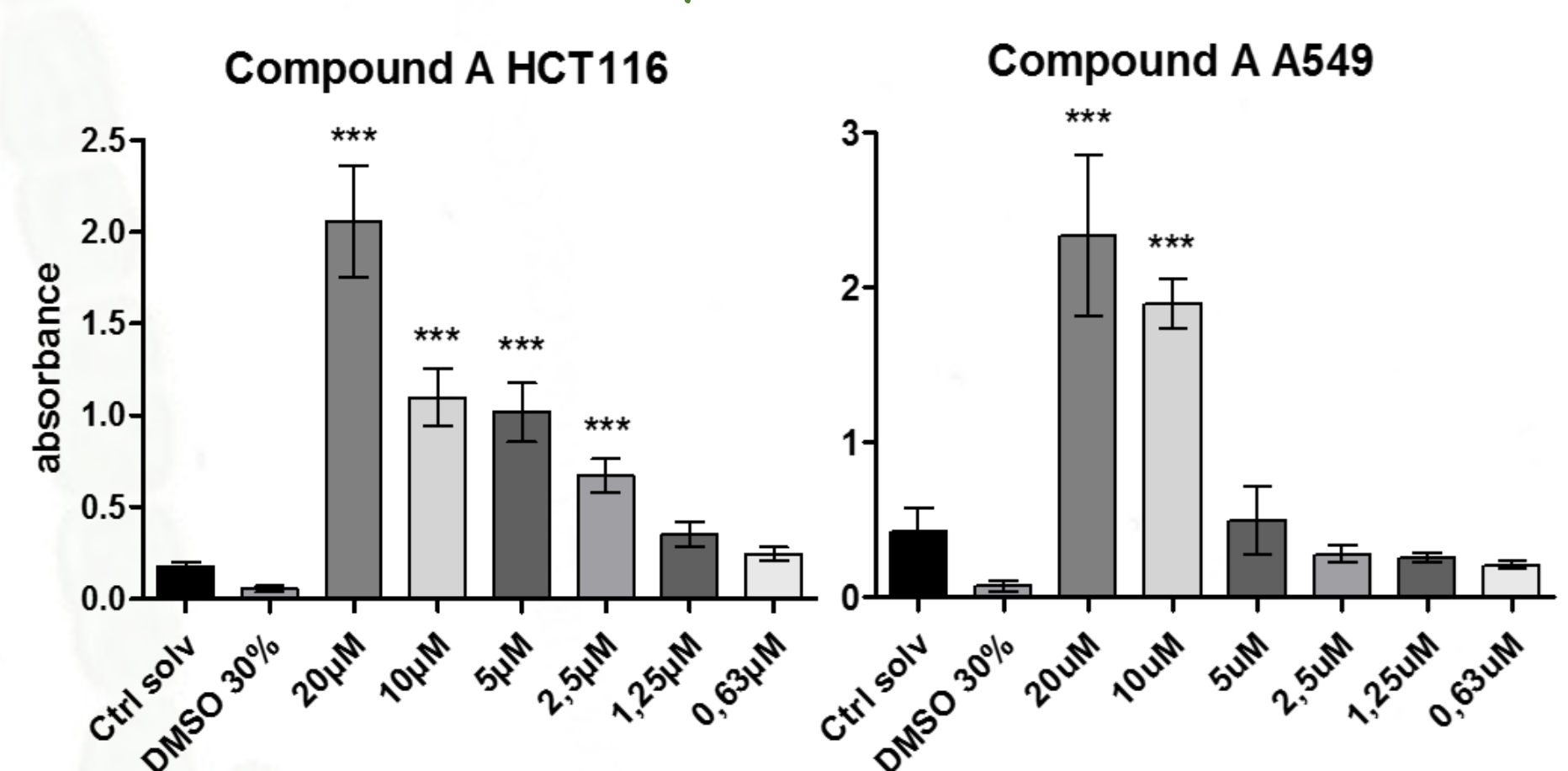


M30 Cytodeath ELISA



Quantification M30 ELISA of apoptosis on spheroids from HCT116 and HT29 cells exposed to Portoamides AB.

M30 Cytodeath ELISA

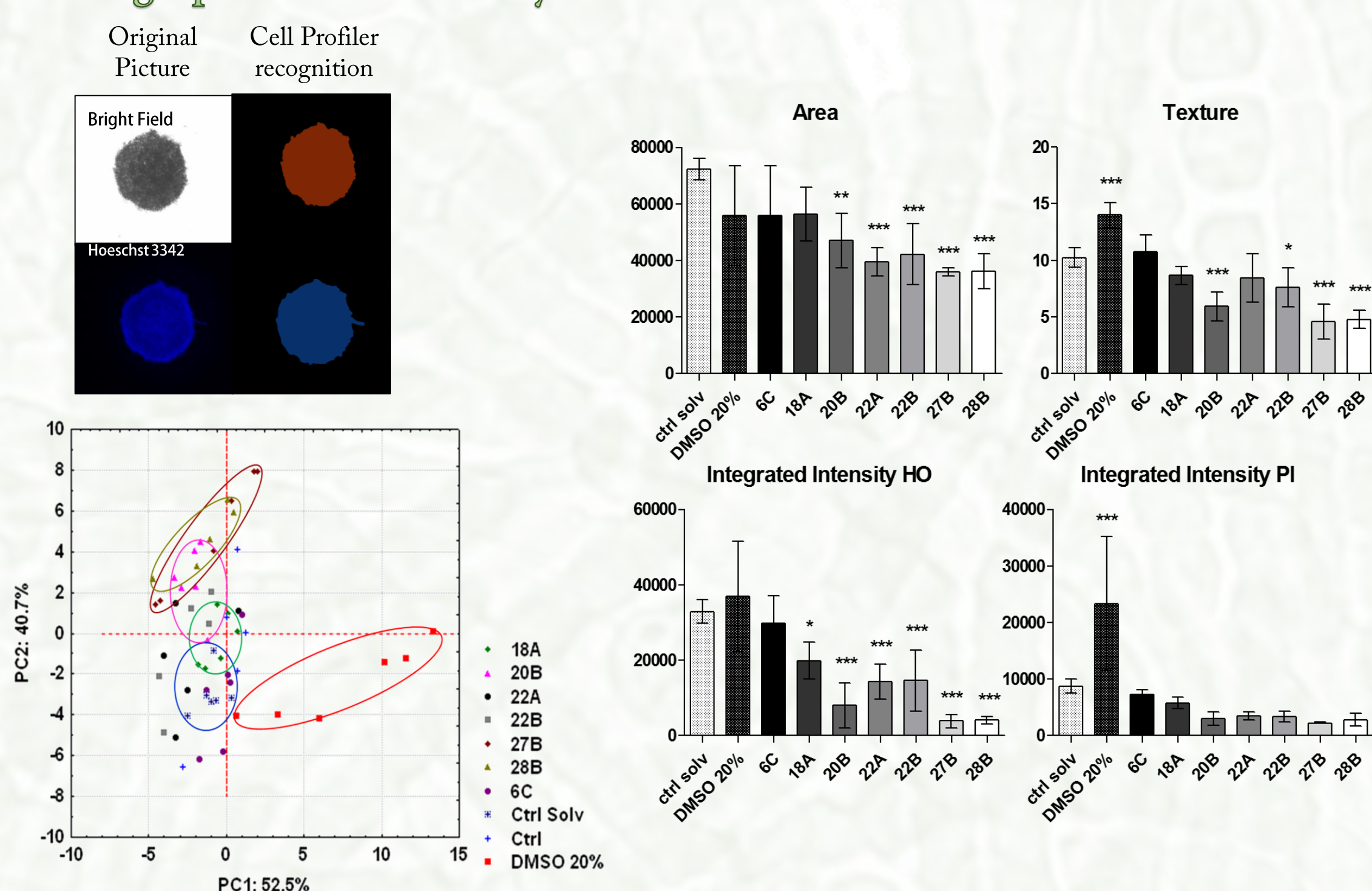


Quantification M30 ELISA of apoptosis on spheroids from HCT116 and A549 cells exposed to Compound A.

Also, Size matters...

Quantitative measure of spheroids area reveal that **Portoamides AB and Compound A increased** significantly their size whereas **Bartoloside B** induced the **decreasing of the spheroids area** (n>3; 2way-ANOVA with Bonferroni post test).

Chromatographic fractions of Cyanobacteria extract



Automated quantification of size of spheroid and nucleus fluorescence were performed using CellProfiler software. In total, 105 parameter were tested, PCA performed on 31 parameter that contributed >0.85 to data variability.

Methods

- Incubation of spheroids on **IncuCyte® ZOOM System** with the different **compounds or fractions of the extracts**.
 - Portoamides A and B** (3:1) mixture, isolated from *Oscillatoria* sp. LEGE 05292
 - Bartoloside B** isolated from and *Synechocystis salina* LEGE 06155
 - Compound A** isolated from *Nodosilinea* sp. LEGE 06071
 - 92 fractions** from 5 different strain of cyanobacteria
- Visualization on **fluorescent microscopy** (Hoeschst 3342 and Propidium Iodide) and **Cell profiler** image analysis.
- An **ELISA M30 Cytodeath assay**
- Qualitative analysis of effects of extracts and selection of seven fractions

Conclusions

- Portoamides and Compound A induce the outer cells to disaggregate
- Bartoloside B decreased the size of HCT116 spheroids
- Portoamides and Compound A induced apoptosis on HCT116 and HT29 spheroids and HCT116 and A549 spheroids, respectively.
- Seven fractions disintegrated the spheroids and from them four fractions (28B, 27B, 20B, 18A) altered consistently the morphology of the spheroid; isolation of the active components will be done in the future by bioassay-guided fractionation.

Tested compounds and extracts show biological activities on 3D spheroids and are therefore interesting candidates to be further developed as therapeutics for solid tumours.