Plant cells viability measurement: spectrofluorometric approach

Jefrem Schwab, Rasha Bassam and Ilya Digel

Institute for Bioengineering
Aachen University of Applied Sciences
D-52428 Jülich, Germany
E-Mail: jefrem.schwab@alumni.fh-aachen.de

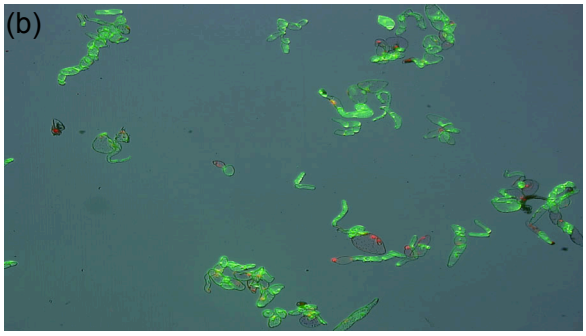
Abstract – In this study we evaluated a new approach for non-invasive viability measurement of suspended plant cells (*Solanum jasminoides* callus tissue) using their intrinsic (natural) fluorescence. Many currently used reagent-aided methods are based on detection of cellular metabolic activity, such as enzymatic hydrolysis of fluorogenic substrates or physiological redox-reactions. For example, esterases can hydrolyze a fluorogenic substrate, namely fluorescein diacetate (FDA) [6] and stain viable cells with the highly fluorescent product fluorescein or in the case of reductases, 2,3,5-triphenyl tetrazolium chloride (TTC) and 3-2,5-diphenyl tetrazolium bromide (MTT) can be converted to formazan upon fully active mitochondria and hence stain viable cells [8].
Another group of methods probe primarily the physical properties of cells, such as cytoplasmic streaming or membrane integrity [1]. In order to assay the membrane integrity, dyes like trypan blue [2], neutral red [3], methylene blue [4], Evans blue [5], phenosafranine [6], propidium iodide [7], SYTOX dyes [7] are widely used. These dyes cannot pass through a viable cell membrane but can leak through disrupted membranes and subsequently stain specific internal contents, such as DNA of non-viable cells (=dye exclusion method). After staining, the viability of the cells can be estimated via a (fluorescence) microscope or a spectrometer.
Although these quantitative viability measures are used in laboratories worldwide and are considered to be the gold-standard, yet none of them has been adopted as optimal and they have still limitations whenever only a small amount of cell probe is available or the method should be non-invasive. Sharon Sowan were among the first researchers who developed a noninvasive measurement of cell viability based on infrared spectroscopy in order to understand “viability” in biochemical terms. They found out that the level of intracellular CO_2 production could be used as a viability determinant for plant cells. The measurement of CO_2 level could be carried out by cooling the suspended cells to subzero temperatures and allowed estimation of viability using infrared spectroscopy [9].
In our study, the idea was to make use of autofluorescent molecules within cells, such as nicotinamide adenine dinucleotide (NADH), flavin adenine dinucleotide (FAD) and others, which reflect mitochondrial activity and thus could be used as natural “viability markers” [10]. For the measurements, living *Solanum* callus cells (1 g) were first suspended in 0.9 % NaCl solution and used to produce a series of dilutions. The overall fluorescence spectrum of the cells was measured with the spectrofluorometer Jasco FP-8500 upon applying the excitation range of 200-350 nm and the emission range of 250-400 nm with a bandwith of 5 nm. A sample with dead cells was prepared by cooking (99°C) the living cells for max. 2 min. In order to achieve different cell viabilities, living and dead cells were adequately mixed. For comparison purposes cell viability was in parallel also quantitatively estimated using PI/FDA assay and fluorescence microscope observation.
Initial results of spectrofluorometric measurement showed, that with this method it is possible to distinguish between 100 % living cells and 100 % dead cells (see Fig. 1) by using reference data. As can be clearly seen in Fig. 1 (a) and (c), the intensity maxima values are shifting depending on the viability of the cells. But at this stage is still not possible to announce a precise viability value. Further experiments have to be conducted in order to eliminate all possible artefacts.
Other possible approaches would be the use of a viability index or the implementation of neural network technology.

(a)

Emission [nm] \ Excitation [nm]	220	225	230	235	240	245	250	255	260	265	270	275	280
300	1390	1786	1253	570	182	115	180	341	487	637	892	1250	1117
305	1469	1902	1586	971	366	199	217	483	792	1162	1560	1228	1496
310	1941	2073	2130	775	392	279	363	500	929	974	1639	1822	1725
315	2173	2067	2194	1157	377	347	490	729	709	1361	1855	2764	2487
320	2252	2553	1353	984	452	545	538	561	1050	1395	1713	3678	3869
325	2226	2715	2158	1001	600	675	707	866	767	1259	1651	4191	2639
330	2541	2693	4411	1290	655	501	518	587	1213	957	2151	3479	2161
335	2415	2599	3668	1532	597	267	431	350	1517	1075	2197	4441	3717
340	2039	2387	1983	1075	578	286	496	597	1647	1159	2655	2360	2287
345	2578	1600	2538	836	743	359	478	538	980	1314	1513	1760	1663
350	1997	2311	1921	596	447	531	526	862	866	1508	992	1555	1937

(c)

Emission [nm] \ Excitation [nm]	220	225	230	235	240	245	250	255	260	265	270	275	280
300	1678	1841	1317	508	171	112	151	257	421	677	956	1198	972
305	1721	1940	1413	570	219	148	174	298	475	741	1003	1205	1048
310	1647	1891	1466	570	270	189	184	317	503	815	931	1256	1237
315	1851	1963	1443	557	319	235	251	321	554	825	1024	1536	1314
320	2391	2345	1666	670	303	286	312	405	614	938	1150	1600	1455
325	2852	2728	1797	603	368	294	355	462	756	1112	1251	1570	1456
330	2515	2609	1640	618	386	295	335	607	788	1075	1245	1446	1610
335	2502	2549	1581	634	438	371	385	482	744	967	1192	1576	1507
340	2473	2408	1601	652	400	404	386	463	725	1069	1045	1405	1401
345	2092	2016	1422	629	343	396	342	384	675	891	903	1092	1330
350	1818	1848	1080	607	328	378	328	386	636	775	897	1099	1162



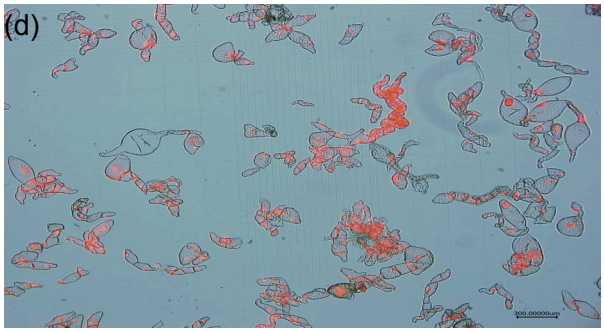


Fig.1: (a) Is showing the spectrofluorometric data as intensity values of approx. 100 % viable cells, shown in (b) as FDA/ PI staining (green= living cells, red= dead cells). (c) Is showing the spectrofluorometric data as intensity values of 100 % dead cells, which is shown in (d) as FDA/PI staining.

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