

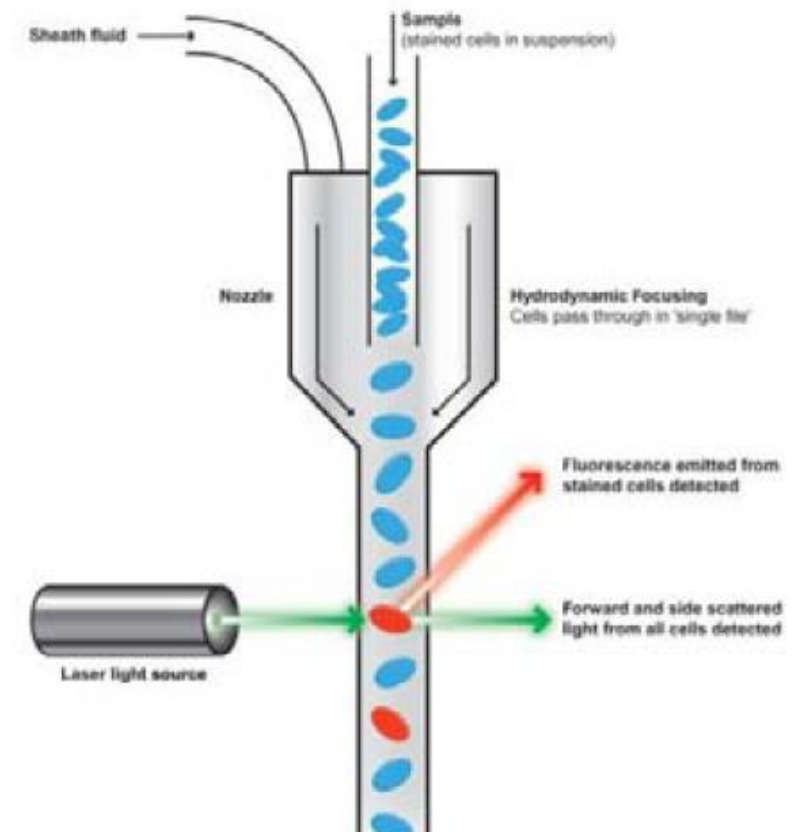
Análise de DNA por Citometria de fluxo

TLAB, 4/10/2022

Pedro Talhinhos

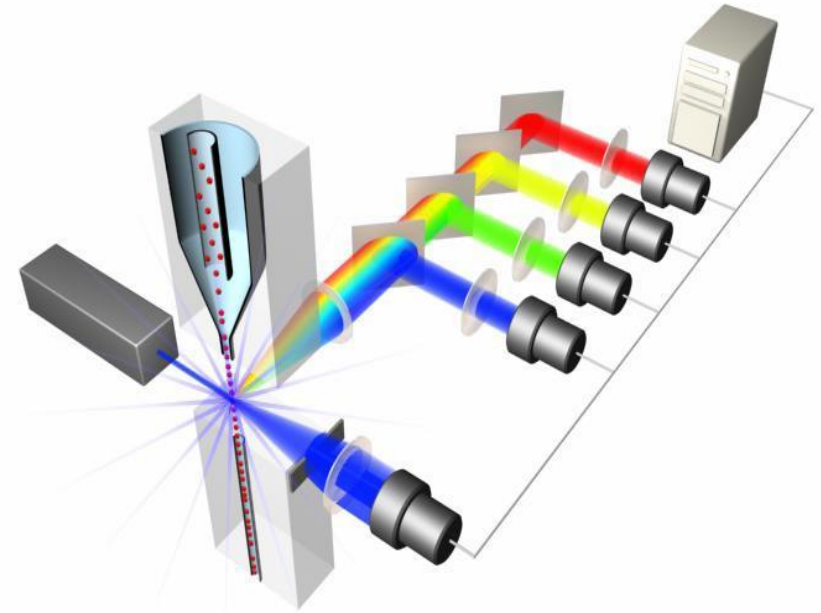
Citometria de fluxo

- Mede propriedades físicas e químicas de células (e outras partículas) transportadas (fluxo) por uma suspensão líquida
- Utiliza um instrumento denominado Citómetro de Fluxo



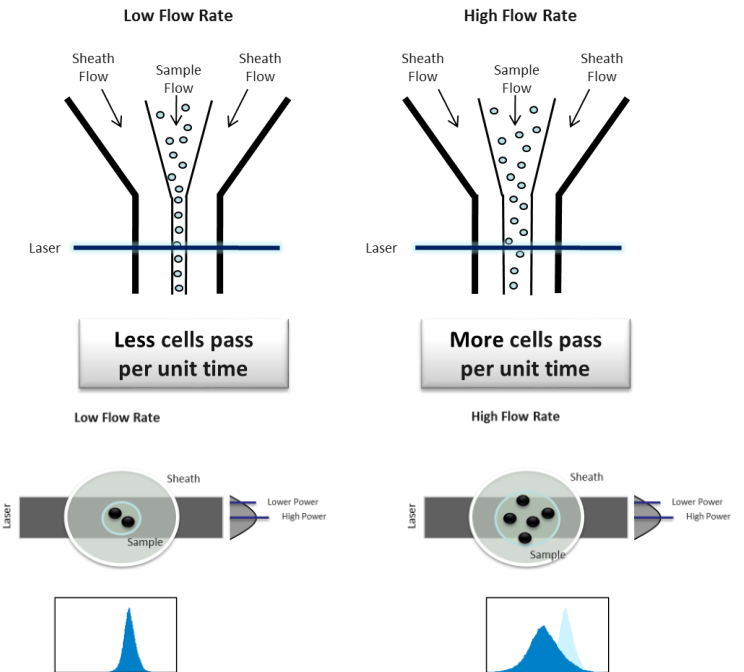
Componentes do citómetro

- Fluídica – assegura que as partículas em análise sejam lidas uma a uma no ponto de interrogação
- Ótica – emissão e leitura de radiação
- Eletrónica – processamento, armazenamento e análise dos resultados da leitura de radiação



Componente fluídica

- A suspensão de partículas (previamente preparada pelo operador) é aspirada e encaminhada e envolvida por um fluxo de líquido (líquido de embainhamento; “sheath fluid”)
- Devido a um fenómeno físico (focagem hidrodinâmica) as partículas são forçadas a mover-se individualmente no centro do fluido
- As partículas são lidas a velocidades de 0,5 a 5,0 μl por segundo (controlável pelo operador)



Componente ótica

Lasers / LEDs

BLUE LASER
488 nm (50 mW fixed/
adjustable to 200 mW)

RED LASER
638/640 nm (25/40 mW)

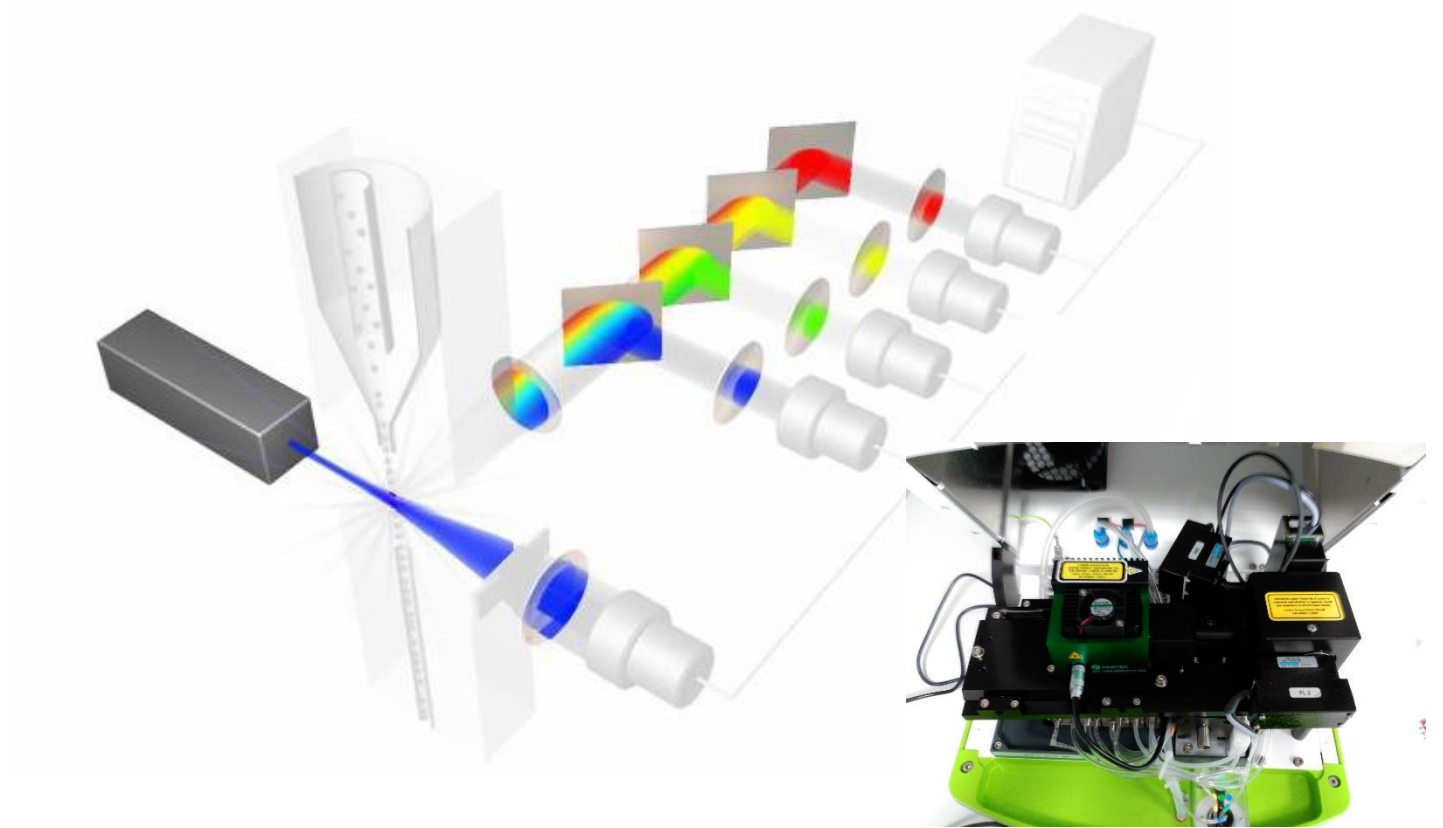
VIOLET LASER
405 nm (100 mW)

UV LASER
375 nm (60 mW)
HIGH-POWER UV LED
365 nm

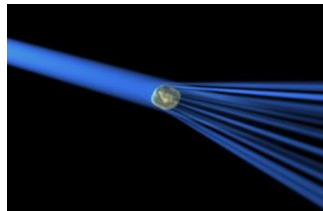
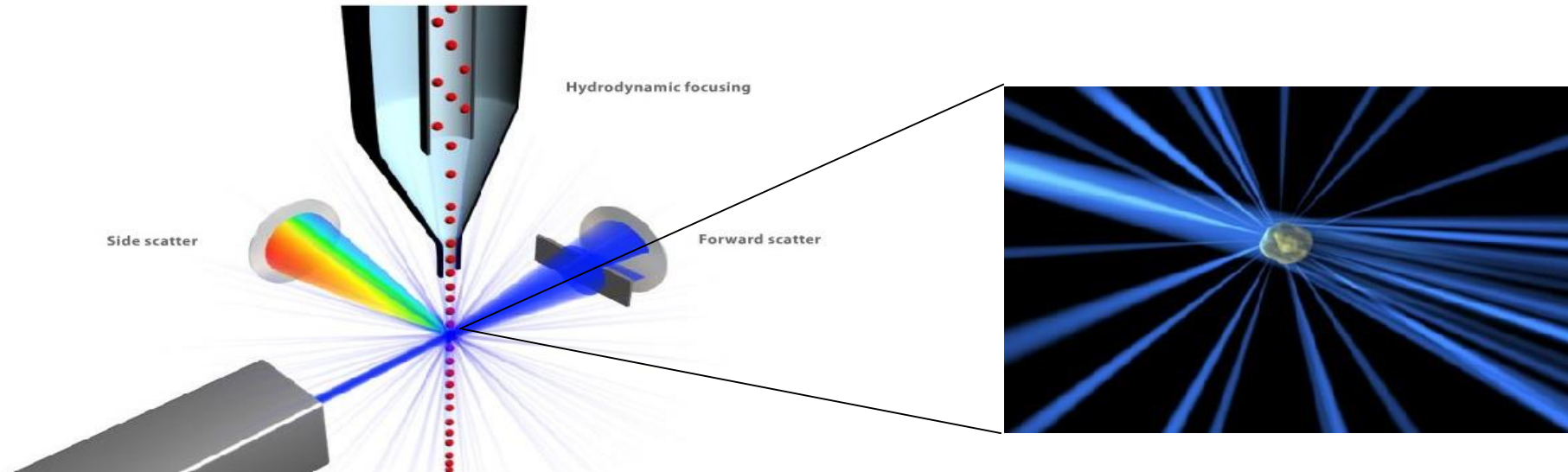
GREEN LASER
532 nm (30 / 100 mW)

YELLOW LASER
561 nm (100 mW)

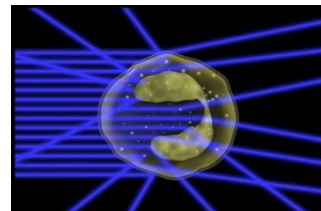
ORANGE LASER
594 nm (50 mW)



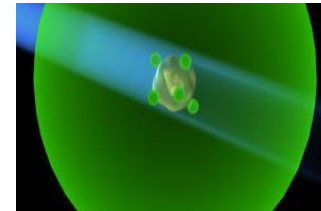
Componente ótica



Forward scatter
Difusão frontal da luz



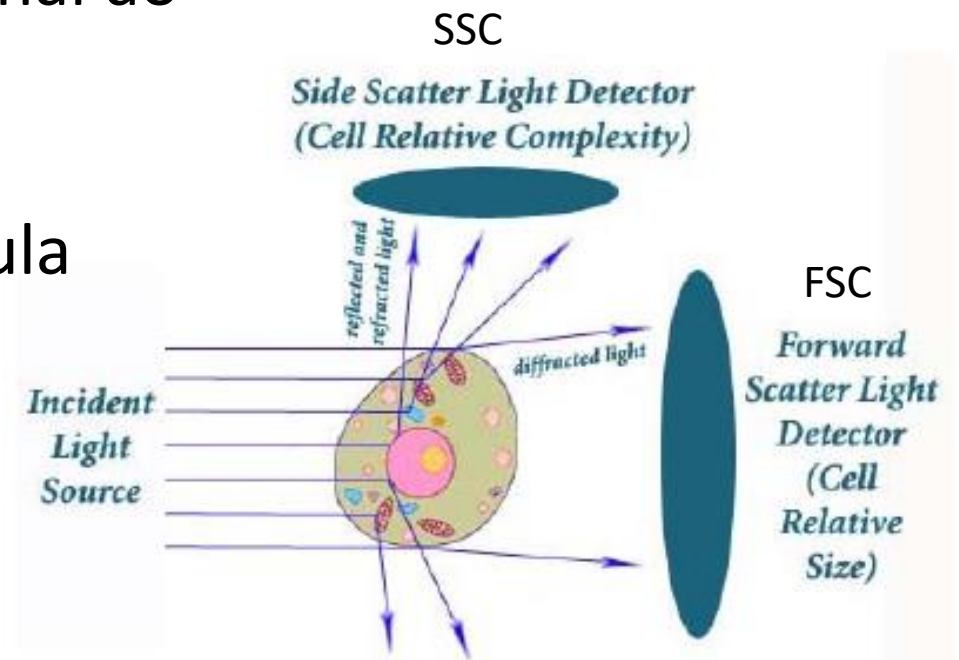
Side scatter
Difusão lateral da luz



Fluorescência
(no caso de marcação com
compostos fluorescentes)

Propriedades mensuráveis

- Difusão frontal da luz (FSC) – proporcional ao tamanho da partícula
- Difusão lateral da luz (SSC) – reflete a complexidade/granulosidade da partícula
- Fluorescência (FL)

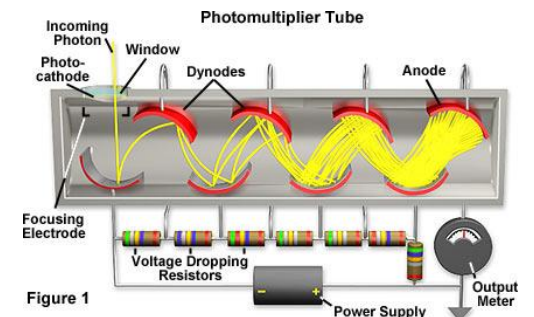


Componente ótica

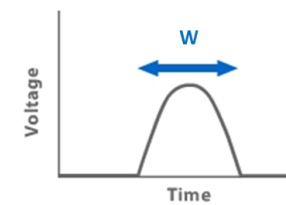
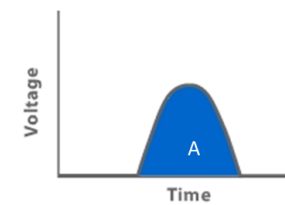
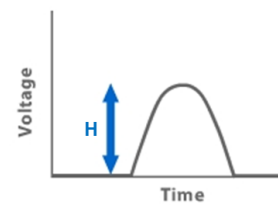
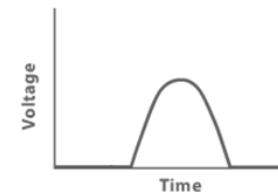
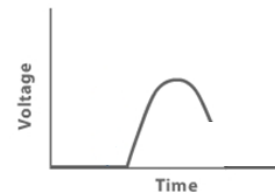
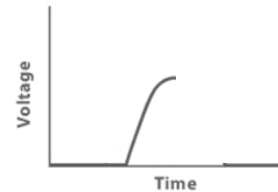
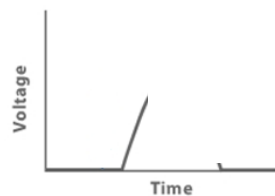
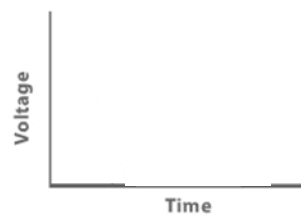
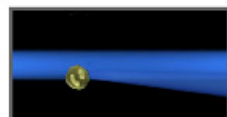
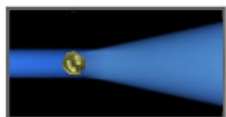
- Os conjuntos de luz difundida e de fluorescência emitida são encaminhados por lentes e espelhos através de **filtros**

permitem dividir a fluorescência em várias cores, possibilitando assim a medição de vários parâmetros numa só amostra

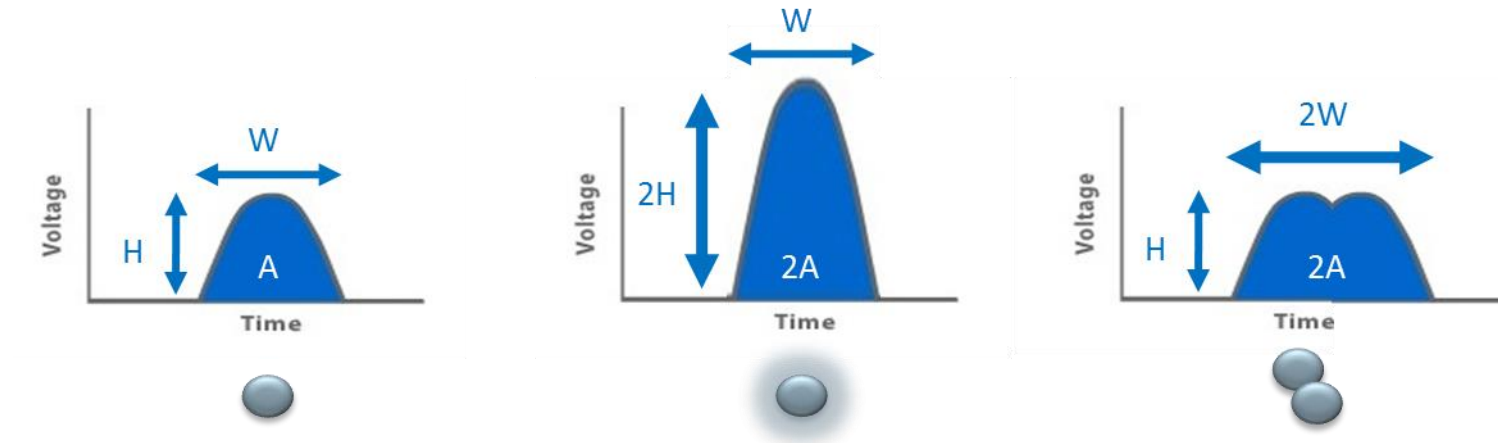
- ➔ para detetores (**tubos fotomultiplicadores**) que amplificam (ganho) e transformam os pulsos de luz em pulsos de corrente elétrica e permitem a passagem do sinal analógico a digital, para posterior processamento e análise computacional



Análise do sinal



Análise do sinal – discriminação de dupletos

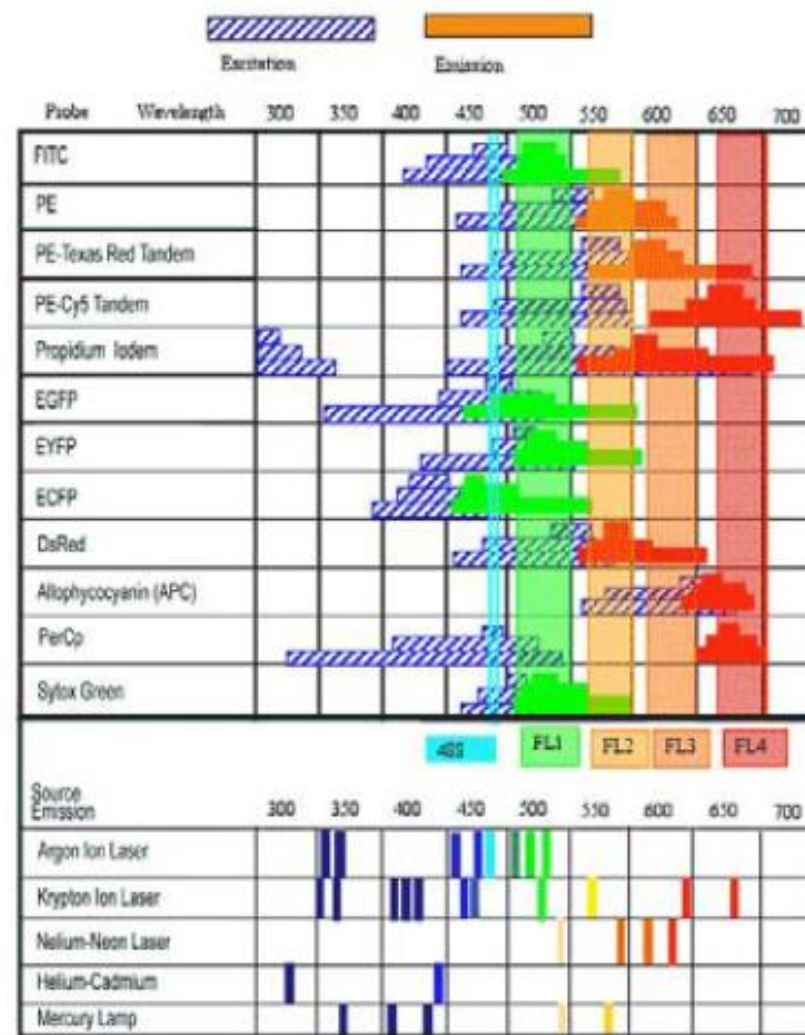


Fluorescência

- propriedade que uma molécula apresenta de absorver luz com um determinado λ e reemitir luz com um λ mais longo (energia mais baixa - Lei de Stokes).

Fluorocromos mais usados em citometria de fluxo e respectivos λ .

Fontes de luz mais usadas

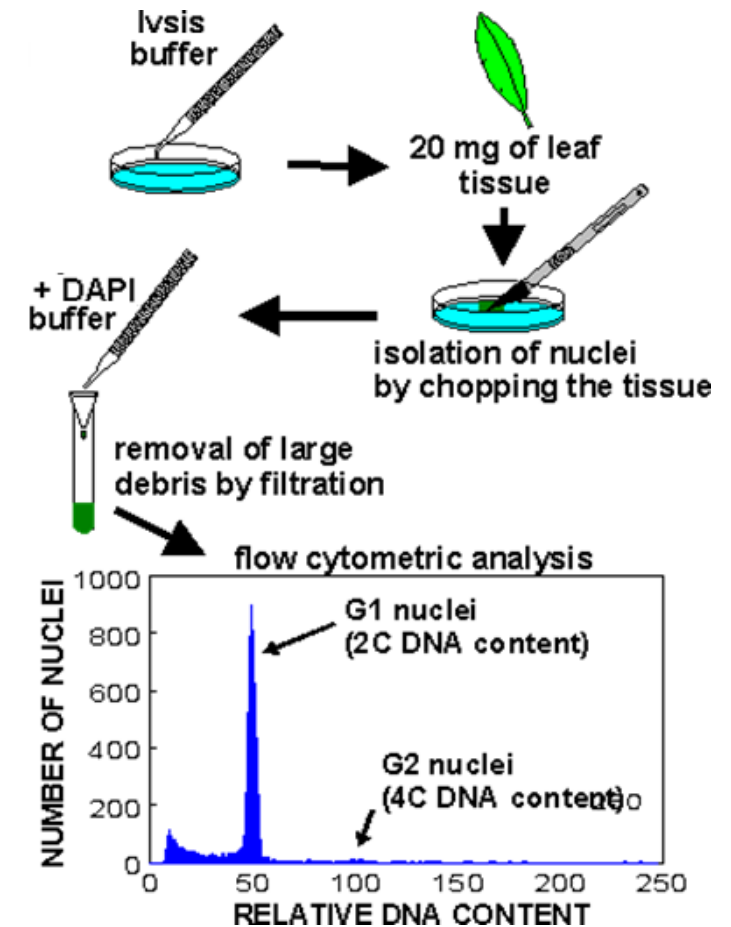


Análise de DNA

- DAPI (4-6-diamidino-2-fenil-indole)
 - Menos sensível a metabolitos secundários
 - Não se liga ao RNA
- Iodeto de propídio
 - Excitação ótima pelo laser verde (532 nm)
 - Liga-se estequiometricamente ao DNA

Preparação da amostra

- Cortar finamente a amostra (lâmina de barbear) libertando os núcleos para a solução tampão (WPB*)
- Remover resíduos grosseiros (filtro 30 μm)
- Corar com PI e homogeneizar
- Alimentar a amostra ao aparelho

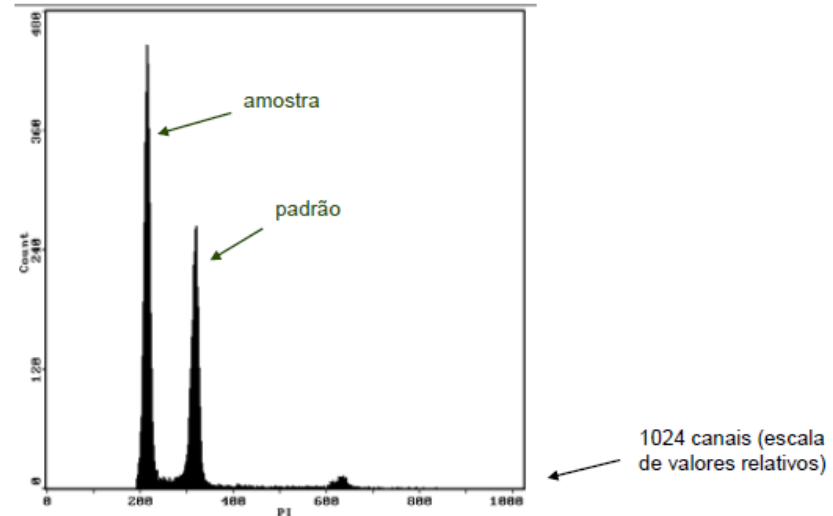


Leitura dos resultados

- Tamanho de genoma – é necessário incluir um padrão de tamanho de genoma conhecido

Interpretação dos resultados

- O eixo x do histograma apresenta uma escala relativa e linear.



Padrões vegetais

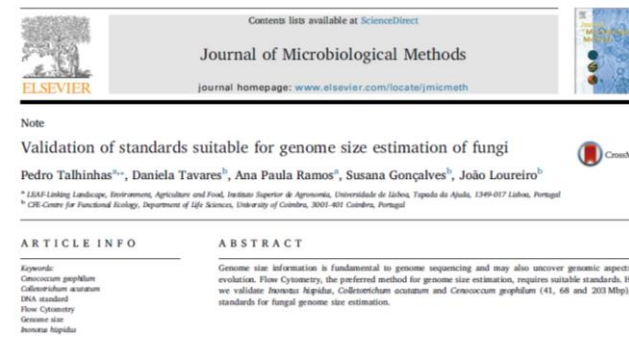
List of DNA standards suitable for plant DNA flow cytometry

Species	Cultivar	2C DNA Content (pg)	1C Genome Size (Mbp)	Reference
<i>Raphanus sativus</i>	Saxa	1.11	543	Doležel et al. (1998)
<i>Solanum lycopersicum</i>	Stupické polní rané	1.96	958	Doležel et al. (1992)
<i>Glycine max</i>	Polanka	2.50	1 223	Doležel et al. (1994)
<i>Zea mays</i>	CE-777	5.43	2 655	Lysák and Doležel (1998)
<i>Pisum sativum</i>	Ctirad	9.09	4 445	Doležel et al. (1998)
<i>Secale cereale</i>	Dankovske	16.19	7 917	Doležel et al. (1998)
<i>Vicia faba</i>	Inovec	26.90	13 154	Doležel et al. (1992)
<i>Allium cepa</i>	Alice	34.89	17 061	Doležel et al. (1998)

Padrões para fungos

List of DNA standards suitable for fungal DNA flow cytometry

Species	1C DNA Content (pg)	1C Genome Size (Mbp)	Reference
<i>Inonotus hispidus</i>	0.0422	41	Talhinhas et al. (2017)
<i>Colletotrichum acutatum</i>	0.0689	67	Talhinhas et al. (2017)
<i>Cenococcum geophilum</i>	0.208	203	Talhinhas et al. (2017)
<i>Rhizoctonia alaternus</i>	0.680 (2C)	664 (2C)	Carvalho et al. (2018)



Fungal genome sizes range from 2.19 Mbp, for the Microsporidia *Encephalitozoon romaleae*, to 3706 Mbp, for the Pezizales *Jaegeria amara* (Egertová and Seehor, 2017). Also, genome sizes vary across fungal phylogeny: while groups such as the Saccharomycotina, the Taphrinomycotina and the Ustilaginomycotina present average genome

30 Pucciniales species (rust fungi), depicting very large genomes in this fungal group. Pires et al. (2016) also used a plant species, *Arabidopsis thaliana* Col-0 (0.32 pg/2C) as standard to determine the genome size of the Ascomycota fungi clustering in the *Colletotrichum gloeosporioides* species complex, although this standard did not perform exceptionally

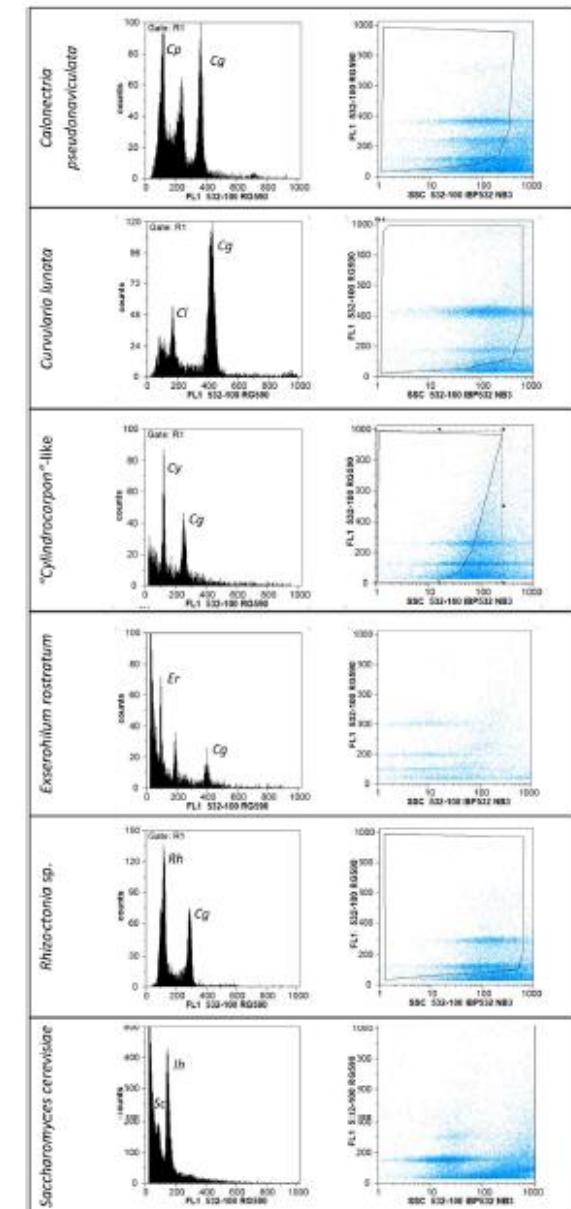


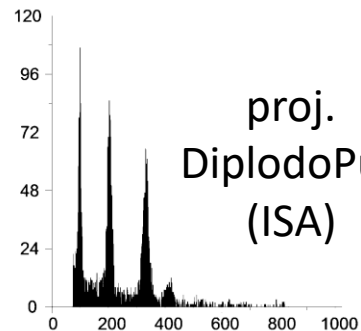
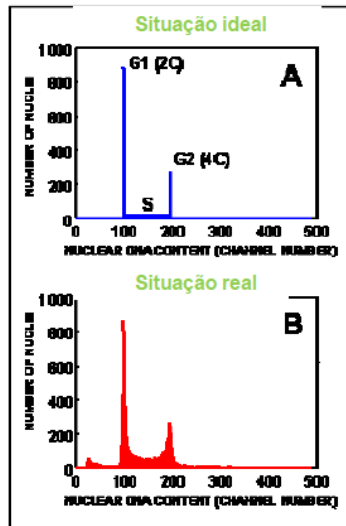
Fig. 2. Genome size measurement results for *Calometria pseudonaviculata* (Cp; 0.0646 pg/1C), *Curvularia lunata* (Cl; 0.0411 pg/1C), '*Cylindrocarpum*'-like (Cy; 0.1003 pg/1C), *Exserohilum rostratum* (Er; 0.0505 pg/1C), *Rhizoctonia* sp. (Rh; 0.0864 pg/1C), *Saccharomyces cerevisiae* (Sc; 0.0103 pg/1C).

Vantagens da citometria

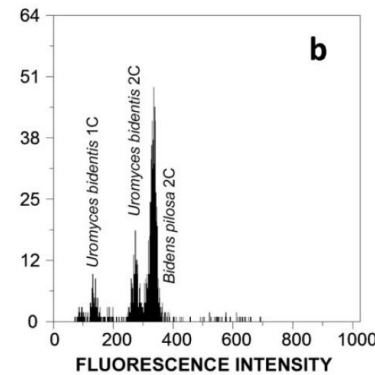
- Medições efetuadas em partículas individuais, mas num grande número de partículas em pouco tempo
- Possibilidade de medir múltiplos parâmetros individualmente em cada partícula
- Possibilidade de recolha de partículas que obedecem a determinadas combinações de critérios (seriadores celulares; “cell sorters”)

Principais aplicações em biologia

- Ciclo celular



- Tamanho do genoma



Molecular Plant Pathology
Molecular Plant Pathology (2015) 14(10), 1026–1030
DOI: 10.1111/mpp.12235

Short Communication

Flow cytometry reveals that the rust fungus, *Uromyces bidentis* (Pucciniales), possesses the largest fungal genome reported—2489 Mbp

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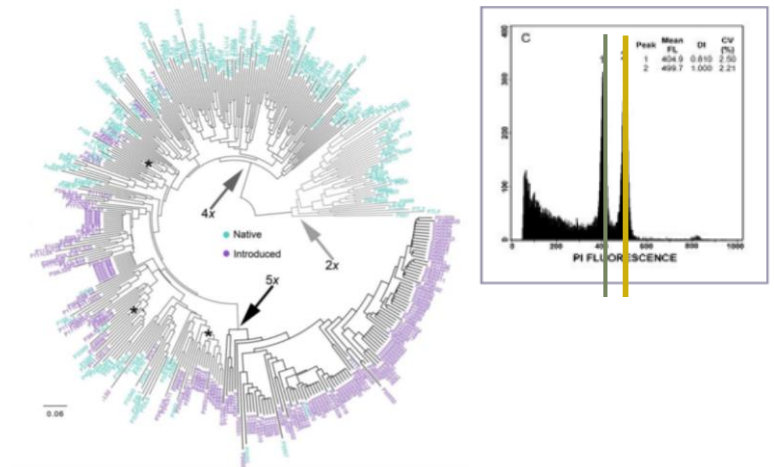
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SUMMARY

Among the Eukaryotes, fungi have relatively small genomes (average of 46.2 Mbp across 1550 species). The order Pucciniales (Basidiomycota) has the largest average genome size among fungi (2489 Mbp).

(Basidiomycota, Pucciniales) have been recognized as the order with the largest average genome size (305 Mbp) (Tavares et al., 2014), with species with genomes as large as 806.5 and 883.2 Mbp (*Puccinia chrysanthemi* Race and *Cymosporangium confusum* Dietel, respectively; Tavares et al., 2014). Genome

- Ploidia



MOLECULAR ECOLOGY

Molecular Ecology (2015) 24, 2143–2155

doi: 10.1111/mec.13006

INVASION GENETICS: THE BAKER AND STEBBINS LEGACY

Invasion genetics of the Bermuda buttercup (*Oxalis pes-caprae*): complex intercontinental patterns of genetic diversity, polyploidy and heterostyly characterize both native and introduced populations

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Abstract

Genetic diversity in populations of invasive species is influenced by a variety of factors including reproductive systems, ploidy level, stochastic forces associated with colonization and multiple introductions followed by admixture. Here, we compare genetic variation in native and introduced populations of the clonal plant *Oxalis pes-caprae* to investigate the influence of reproductive mode and ploidy on levels of diversity. This species is a tristylous geophyte native to South Africa. Invasive populations throughout much of the introduced range are composed of a sterile clonal pentaploid short-styled

Tamanho do genoma

- Como forma de medir a diversidade genética numa espécie



Article

Genetic and Genomic Diversity in a Tarwi (*Lupinus mutabilis* Sweet) Germplasm Collection and Adaptability to Mediterranean Climate Conditions

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Received: 7 October 2019; Accepted: 20 December 2019; Published: 22 December 2019



Abstract: *Lupinus mutabilis* (tarwi) is a species of Andean origin with high protein and oil content and regarded as a potential crop in Europe. The success in the introduction of this crop depends in part on in depth knowledge of the intra-specific genetic variability of the collections enabling the



Table 6. Genome size of the *Lupinus mutabilis* accessions estimated by flow cytometry.

Accession	Genome size (Mbp)		H.G. ²
	average	StDev ¹	
SBP	1897.3	18.4	a
XM1-39	1907.3	17.0	a
JKI-L378	1938.0	49.0	ab
Prt-79	1957.4	16.2	ab
JKI-L377	1961.4	16.3	ab
Mutal	1967.6	121.6	abc
JKI-L295	1969.0	11.8	abc
JKI-L210	1973.6	26.9	bcd
LM13	1975.7	118.4	bcd
JKI-L309	1979.7	37.6	bcd
LM231	1984.1	100.4	cd
LM18	1986.4	54.6	cde
XM5	2009.1	20.1	cde
P-20993	2021.5	22.8	cde
Potosi-ISA	2024.3	36.1	cde
Potosi-ALE	2024.8	19.3	cde
LM27	2027.7	23.9	cde
LM32	2040.9	17.9	de
CM157	2040.9	43.8	de
I82	2041.6	10.9	de
Inti	2058.1	23.9	ef
LM268	2078.9	10.1	f
LM81	2080.2	13.1	f
LM34	2083.2	17.3	f

¹ Standard deviation; ² Homogeneous Groups – accessions sharing the same letter are not statistically different.

9,2%
variabilidade
no tamanho
do genoma



Article

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9,2%
variabilidade
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do genoma

scientific reports

OPEN

Exploring physicochemical and cytogenomic diversity of African cowpea and common bean

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In sub-Saharan Africa, grain legumes (pulses) are essential food sources and play an important role in sustainable agriculture. Among the major pulse crops, the native cowpea (*Vigna unguiculata*) and introduced common bean (*Phaseolus vulgaris*) stand out. This paper has two main goals. First, we provide a comprehensive view of the available genetic resources of these genera in Africa, including data on germplasm collections and mapping biodiversity-rich areas. Second, we investigate patterns of physicochemical and cytogenomic variation across Africa to explore the geographical structuring of variation between native and introduced beans. Our results revealed that 73 *Vigna* and 5 *Phaseolus* species occur in tropical regions of Africa, with 8 countries accounting for more than 20 native species. Conversely, germplasm collections are poorly represented when compared to the worldwide collections. Regarding the nuclear DNA content, on average, *V. unguiculata* presents significantly higher values than *P. vulgaris*. Also, *V. unguiculata* is enriched in B, Mg, S, and Zn, while *P. vulgaris* has more Fe, Ca, and Cu. Overall, our study suggests that the physicochemical and cytogenomic diversity of native *Vigna* species is higher than previously thought, representing valuable food resources to reduce food insecurity and hunger, particularly of people living in African developing countries.

Leguminosae (nom. alt. Fabaceae family) is characterized by approximately 770 genera and 19,500 species, being largely cultivated worldwide^{1,2}. The crops within this family provide an important resource throughout the tropi-

Phaseolus
vulgaris 7,8%

Vigna
unguiculata
17,6%

Hemileia
vastatrix
21,0%

A high genomic variability is found among *Hemileia vastatrix* pathotypes

Laureano, A.¹, Rodrigues, A.S.³, Macedo, C.^{1,2}, Modesto, I.⁴, Diniz, I.^{1,2}, Várzea, V.^{1,2}, Miguel, C.⁴, Talhinhos, P.², Batista, D.^{1,3}

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Coffee leaf rust is a devastating disease caused by *Hemileia vastatrix* (*Hv*), leading to huge losses in coffee production. There are over 50 races/pathotypes of *Hv*, so it is of great importance to comprehend how the genetic variation is promoted, as well as the regulator mechanisms of the differentiated pathogen virulence profiles. Retrotransposons are associated with genome structural variations and gene expression regulation in plant pathogens. These features may be associated to their capacity to rapidly evolve virulence. In this study, the genome size and the variability of a *Ty1/Copia*-like retrotransposon, concerning nucleotide sequence and copy number, were investigated among *Hv* contrasting pathotypes to assess their putative association

Ploidia



Ciclo celular

