Light scattering measurements for quantifying biological cell concentration: an optimization of opto-geometric parameters

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An experimental study was carried out, aimed at optimizing the opto-geometric configuration for measuring the concentration of biological cells by means of static light scattering measurements. A LED-based optoelectronic setup making use of optical fibers was experimented, as the precursor of a low-cost device to be integrated in instrumentation for cytometry. Two biological sample types were considered as test samples of the most popular analyses - cervical cells and urine, respectively. The most suitable wavelengths and detecting angles were identified, and calibration curves were calculated. [DOI: http://dx.doi.org/10.2971/jeos.2012.12003]

Keywords: light scattering, biological cell concentration, PAP-test, urine

1 INTRODUCTION

Liquid-based cytology (LBC) is an innovative way of preparing biological samples for cytological examinations in the laboratory. It consists of fixing the collected sample in a preservative alcohol-based fluid for further clarification, centrifugation, and then depositing a thin layer of cells on a slide. The ensuing examination is carried out by the cytologist in the usual way under a microscope. Today, LBC is the most widely used form of its kind in applications to gynaecological cervical smears (PAP-test), for which it was originally developed [1]-[3]. Subsequently, LBC has progressively gained favour also in many others cytologies [4], especially urinary [5], oral [6], naso-pharyngeal [7], as well as for breast tumor analysis [8].

Since 1999, Hospitex Diagnostics srl has been implementing an innovative and effective proprietary LBC method, the CYTOfast® system [9]. It makes use of CYTOfast® solution, a universal preservative solution that makes it possible to preserve the physiological structure and morphology of any kind of cell for 24 months at room temperature. Cellular material left in the vial, after the slide preparation, can be used directly for further investigations employing molecular biology techniques (e.g. PCR, hybridization, etc.).

The CYTOfast® system makes use of a *standardization phase* during which a nephelometric reading determines the cellular density of the samples. In accordance with this idea, the system fixes the quantity to add to the slide for every sample, in order to obtain numerically standardized slides, which always contain the same number of cells, distributed as a monolayer, on a spot having a diameter of 17 mm, for a safer, faster, easier and representative screening (approximately 100.000 cells). The better the knowledge of the cellular density, the better the monolayer uniformity and quality and, consequently, the results of cytological analyses will be.

Static light scattering has long been a standard method for cell concentration assessment in biological samples [10]. This paper presents the results of an experimental nephelometric study which was performed on cervical and urine cells in a CYTOfast® solution. The scope of the experiment was to optimize the opto- geometric configuration for measuring the concentration of biological cells by means of static light scattering measurements. A LED-based optoelectronic setup making use of optical fibers was experimented, as the precursor of a low-cost device to be integrated in instrumentation for cytometric purposes. The most suitable wavelengths and detecting angles were identified, and calibration curves were calculated.

2 Experimental Setup

Transmission and multiple-angle scattering measurements of biological samples were carried out by means of the experimental setup sketched in Figure 1. A glass vial containing 32 ml biological sample was inserted in a jig. Four LEDs were used for illumination at four wavelengths: 405 nm, 525 nm,

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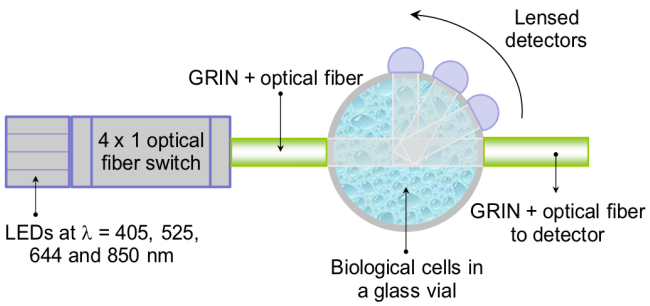

FIG. 1 Diagram of the experimental setup.

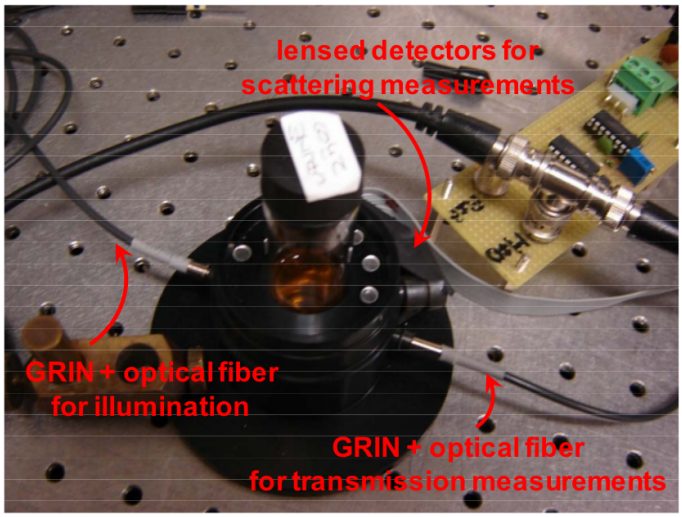

FIG. 2 Practical implementation of the experimental setup.

644 nm, and 850 nm, respectively. The receptacles for LED housing were coupled to a 4 x 1 fiber-optic switch which provided wavelength-sequential illumination to the vial by means of a single optical fiber (HCS type, 200 μm core diameter). This fiber was coupled to a SELFOC® collimator [11], including a graded-index (GRIN) lens, for illuminating the sample by means of a nearly-collimated beam. An identical optical fiber SELFOC® collimator was used for transmitted light detection. Three lensed detectors, directly butt-coupled to jig, were used to measure the scattered light at 30°, 60° and 90°. The jig was designed and precisely mechanical drilled so as to house the SELFOC® collimators and lensed detectors in the proper axial alignment and reciprocal angle positions without the need of any further alignment. Figure 2 shows a view of the practical implementation of the experimental setup.

Cells from PAP-tests and urine were fixed in CYTOfast® solution, and biological samples were prepared that had cell concentrations in the 20-1000 cell/mm^3 range, which typically occur in real conditions. While PAP- test solutions were practically colourless, urine solutions exhibited a yellow colour, ranging from pale to intense, depending on the urine concentration.

PAP test

$\theta(°) - \lambda(nm)$	A	B
30 - 405	2.35 e3	0.70
30 - 525	7.03 e3	0.71
30 - 644	**1.80 e4**	**0.67**
30 - 850	2.90 e4	0.73
60 - 405	5.74 e3	0.56
60 - 525	1.91 e4	0.59
60 - 644	5.62 e4	0.59
60 - 850	1.59 e5	0.68
90 - 405	1.48 e4	0.58
90 - 525	4.53 e4	0.60
90 644	2.10 e5	0.64
90 850	5.82 e5	0.74

Urine

$\theta(°) - \lambda(nm)$	A	B
30 - 405	4.25 e3	0.66
30 - 525	1.16 e4	0.65
30 - 644	3.58 e4	0.66
30 - 850	5.93 e4	0.70
60 - 405	1.38 e4	0.57
60 - 525	**2.92 e4**	**0.54**
60 - 644	1.12 e5	0.58
60 - 850	3.16 e5	0.68
90 - 405	3.52 e4	0.61
90 - 525	8.82 e4	0.58
90 - 644	6.21 e5	0.67
90 - 850	1.56 e6	0.76

TABLE 1 Summary of fitting parameters for PAP-test (upper) and urine (lower) cell solutions.

3 Measurement Results

The entire biological set was optically characterized by measuring the ratio between scattered and transmitted light power, $x = P(\theta)/P(0°)$, at all wavelengths and at all angles. This ratio provided a normalized output that was independent of absorption effects and source intensity fluctuations. Because of multiple scattering phenomena, the relationship between cell concentration and the normalized output was nonlinear. This behavior was satisfactory represented by means of the power law described by;

$$C(x) = Ax^B \tag{1}$$

Table 1 summarizes the fitting parameters for all experimented wavelength-angle combinations, and the best conditions are highlighted in bold. The most efficient angle was found to be 30° at 644 nm for PAP-test and 60° at 525 nm for urine cell solutions. The experimental results and relative fitting functions are shown in Figure 3. Because of their morphological differences, the urine cells exhibited a lower scattering efficiency as compared with the PAP-test cells.

Urine suspensions, thanks to their lower scattering efficiency, were well represented by the power law over the full concen-

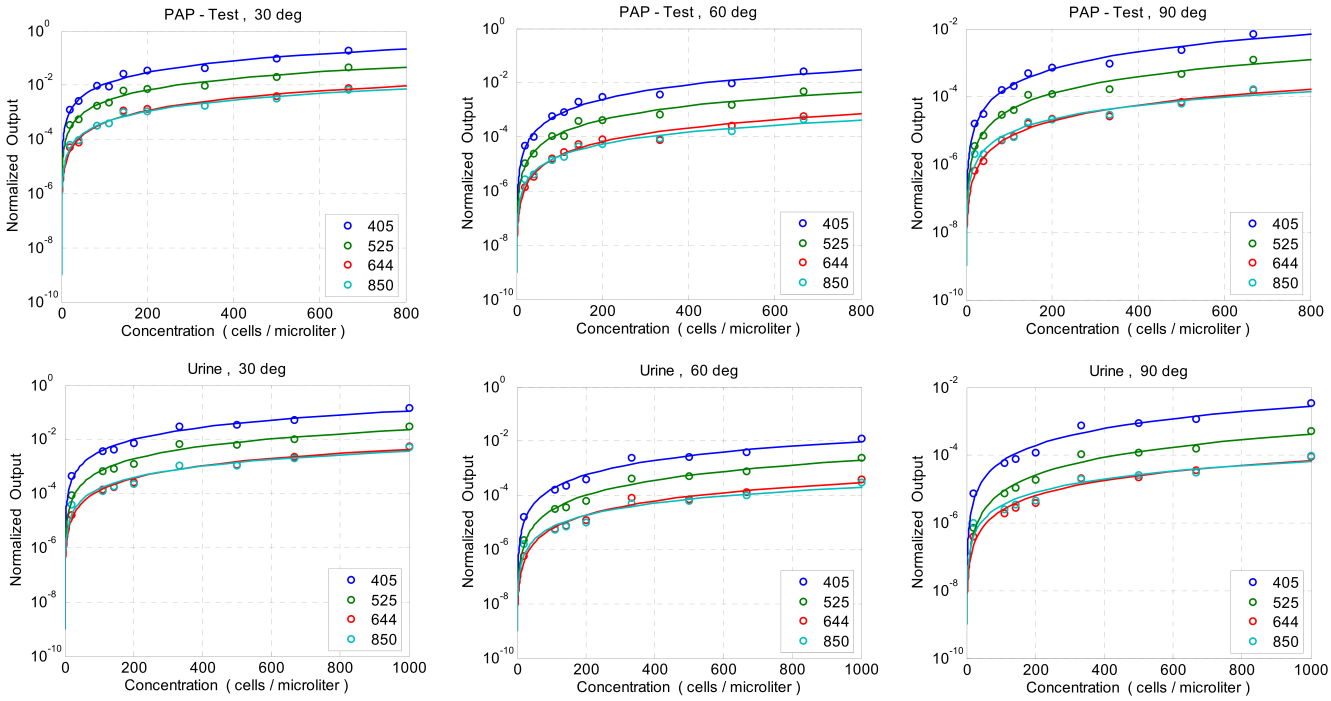

FIG. 3 Experimental results (marks) and fitting functions for PAP-test (left) and urine (right) cell solutions at all angle-wavelengths combinations.

PAP test				
$\theta(^\circ)$	405 nm	525 nm	644 nm	850 nm
30	0.958	0.930	**0.964**	0.954
60	0.942	0.898	0.942	0.881
90	0.907	0.901	0.933	0.814

Urine				
$\theta(^\circ)$	405 nm	525 nm	644 nm	850 nm
30	0.911	0.892	0.917	0.836
60	0.942	**0.947**	0.919	0.815
90	0.937	0.927	0.909	0.775

TABLE 2 Summary of determination coefficient, R^2, of the fitting functions, for PAP-test (left) and for urine (right) cell solutions.

tration range. PAP-test suspensions, because of their higher order multiple scattering, exhibited a good fit up to 700-800 cell/mm^3. The 1000 cell/mm^3 sample was considered out of the validity range, because its prediction resulted underestimated well beyond the evaluated confidence limits.

The goodness of fit was assessed by calculating the determination coefficient, R^2, and the standard deviation of the residuals, σ. The determination coefficient was the squared correlation coefficient between the measured and the predicted concentration values. The closer R^2 to 1, the better the fit. The standard deviation of the residuals was the mean root square value of the differences between the measured and fitting-predicted values. In practice, it measured the average deviation between the two sets of values, and was expressed by means of;

$$\sigma = \sqrt{\frac{(C - C')^2}{N - 2}} \qquad (2)$$

being C the measured concentration, C′ the fitting-predicted concentration, and N the number of calibration points.

Tables 2 and 3 summarize the values of R^2 and σ for all angle-wavelength combinations. Those providing the lowest σ were considered the best, and are highlighted in bold. The goodness of σ could be assessed by comparing it with the concentration values. The percentage prediction residuals for the optimal configuration are shown in Figure 4: most of the samples show prediction residuals within 20%.

4 Perspectives

PAP-test and urine cells fixed in CYTOfast® solution were considered. A comprehensive nephelometric study for cell density measurements was carried out at several illumination wavelengths and detection angles. This made it possible to determine the best wavelength-angle combination for each type of biological cells. Our study was aimed at implementing an automatic device capable of drawing a fixed cell amount to be smeared on a microscope-glass. In fact, dealing with a fixed number of cells enabled us to obtain a uniform and good quality cell monolayer, which is what is needed for optimal LBC cytological analyses.

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PAP test				
$\theta(°)$	405 nm	525 nm	644 nm	850 nm
30	46	59	**43**	48
60	56	59	59	68
90	58	60	64	74

Urine				
$\theta(°)$	405 nm	525 nm	644 nm	850 nm
30	99	109	96	135
60	80	**76**	95	143
90	84	90	100	158

TABLE 3 Summary of standard deviation of residuals, σ, of the fitting functions, for PAP-test (left) and for urine (right) cell solutions.

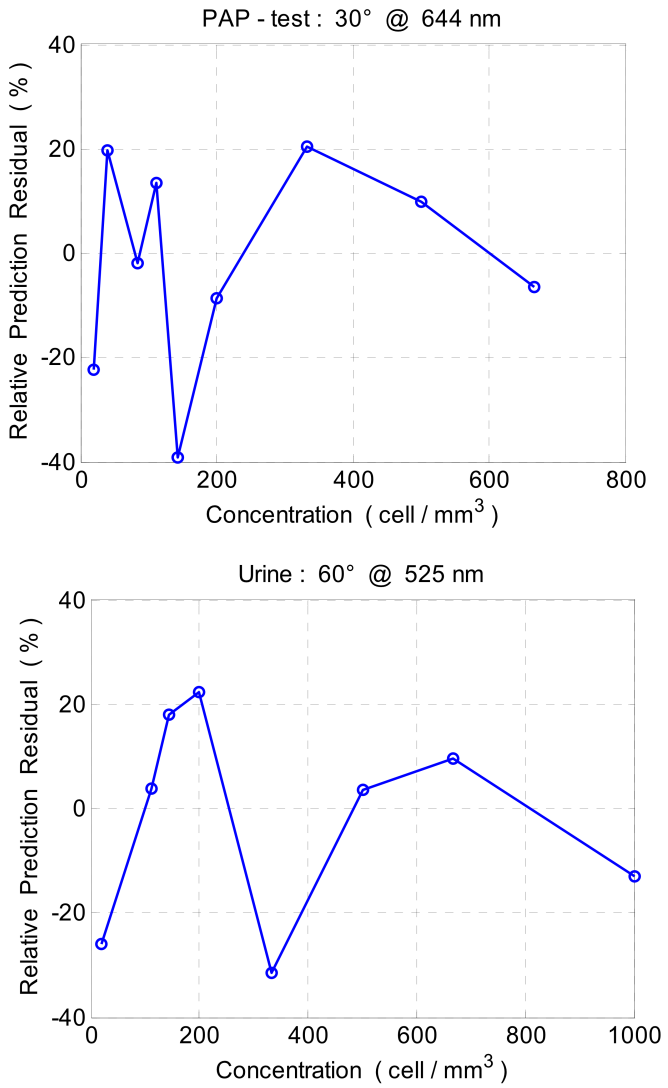

FIG. 4 Behaviour of residuals as a function of cell concentration, for PAP-test (left) and urine (right) cell solutions, at optimal angle-wavelength combinations.

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