Flow cytometry and its application to the pulp and paper industry

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In this paper I aim to give a brief introduction to an analytical technique namely flow cytometry, outlining how it works and providing some insights into its potential application and usage within the pulp and paper industry. Examples provided will hopefully illustrate the usefulness both from the point of view of a chemical supplier, and the client. The focus of this paper will be to show how flow cytometry can be a beneficial tool to gain insight into the health and composition of paper mill water systems, and also how both quantitative and qualitative information can be acquired to demonstrate the efficacy of various treatment regimes.

Overview of history and background

This technique has been around for a surprising number of years. Development in earnest began during the 1950s at a time when the need for automated cell counting within haematology labs was becoming evident. Until that time diagnostics for blood disorders required arduous manual cell counting and characterisation using a microscope and haemocytometer; not only was this a lengthy procedure, it was also prone to error, thus an alternative was required. The first breakthrough came in the form of the coulter counter, an instrument that measured the size of a cell by electrical means as it passes through a very narrow aperture. The measured impedance to electrical current across the aperture being proportional to the cell size, this provided a means to measure the number and size distribution of cells within a sample.[1] It wasn't long before the potential benefits of optical detection were realised, and led to the development of the modern machines that we see today. This process of refinement began with the very first recognisable commercial flow cytometer created by Wolfgang Göhde, and manufactured by Partec in Münster through Phywe AG in Göttingen,[2] and continues to this day with developments such as fluorescence activated cell sorting, and the microfluidic 'lab on a chip' Ampha Z30.

Operation

Figure 1 shows a simplified schematic showing the inner workings of a flow cytometer. The principle of operation is fairly straightforward, with the sample under investigation being introduced into the machine where it becomes distributed in a flow of liquid. This liquid stream is then directed to pass through the focus of a laser beam, any cells or particles within the stream interact with incident laser light and these interactions are measured. Properties that can be directly or indirectly measured include granularity, size, and optical fluorescence.[3]

Since we are measuring multiple parameters simultaneously, a much more detailed picture of the sample population and its inherent properties is obtained when compared to single parameter measurement techniques. From the point of view of the paper industry this becomes more useful when you consider the application of fluorescent markers; indeed one of the most intractable issues facing the paper industry today is that of machine runnability in the face of mounting pressure to close water loops, and reduce total water consumption. A side effect of this is that paper mill water systems have become increasingly dirty, with significant quantities of sticky or pitch-like substances accumulating in the machine water loop, causing not insignificant amounts of downtime for cleaning and removal of deposits from the surfaces of paper machines.[4,5] This problem occurs extensively in virgin pulp mills where the pitch-like materials are principally tree oils and wood extractives; it is also a major disturbance in recycled pulp mills where the interfering substances tend to be a mixture of inks, glues, and coatings from packaging and other waste sources. Knowledge of the fact that most of these process interfering substances have a common characteristic, in that they are generally hydrophobic or lipophilic in character, gives us the ability to make measurements of such components. This insight allows us to utilise a well-known fluorescent marker to selectively stain hydrophobic moieties in samples taken from the paper machine and make direct meas-

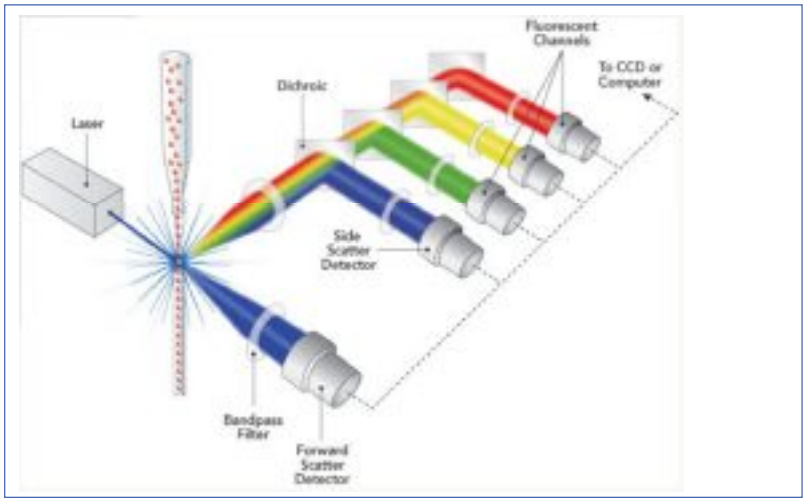


Figure 1. The flow cytometer (Illustration courtesy of Life Technologies Corporation)

urement of the composition and characteristics of any paper machine sample that you care to measure. This marker is Nile red, and was originally used to measure intra/extra cellular fat distribution in biological systems. This marker is solvatochromic, meaning the spectral properties are dependent on the environment that the dye finds itself (*Figure 2*): in non-fatty, polarised solvent environments the fluorescence wavelength is centred quite differently to dye particles in more fatty less polarised environments.

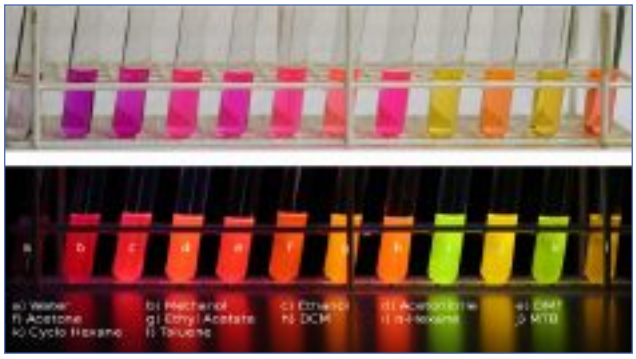


Figure 2. An illustration of the solvatochromic nature of Nile red stain in various environments. Note that the more lipophilic environments fluoresce at shorter wavelengths (golden yellow: n-Hexane) than do less lipophilic (red: Methanol). Also note the almost complete absence of fluorescence in water.

This can be quantified by reference to the chart comparing the fluorescence intensity and the partition coefficients of a selection of solvents, as shown in *Figure 3* and *Table 1*.

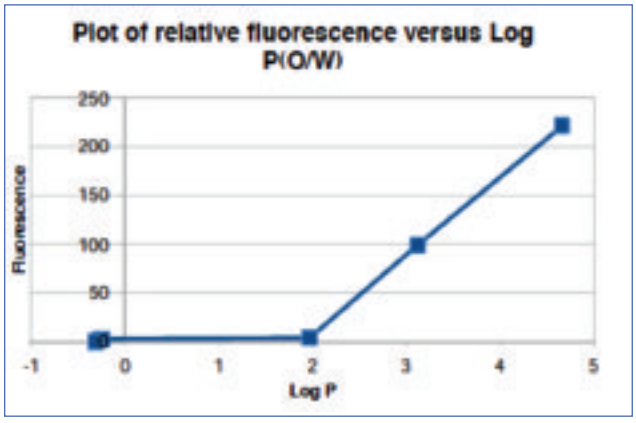


Figure 3. Increasing ocatane:water partition coefficient equates to increasing lipophilic / decreasing hydrophilic property.

	Log P(O/W)	Fluorescence
N-Heptane	4.66	221
2-Xylene	3.12	99
Chloroform	1.97	4
Acetone	-0.24	3
Ethanol	-0.31	0.1

Table 1. Relative fluorescence of Nile red in various solvents.

With this marker we can make assessments of the lipophilic nature of paper mill water phase, and make estimates of the potential process interfering components. However, before we can perform any useful measurements, first we need to calibrate the instrument such that we can make inferences about the size of measured particulates, and we also need to characterise the undesirable components within the mill water systems to prove we are categorising the particulates correctly. To achieve this, we first make measurements of certified particle size standards; these can be purchased as polystyrene beads of known size distribution from laboratory suppliers. These measurements can then be used to construct a curve relating measured parameters (side scatter) to physical size in micrometres (*Figure 4*). Note there is a limit of about 150µm; above that size particulates may block the equipment and invalidate any observations.[7]

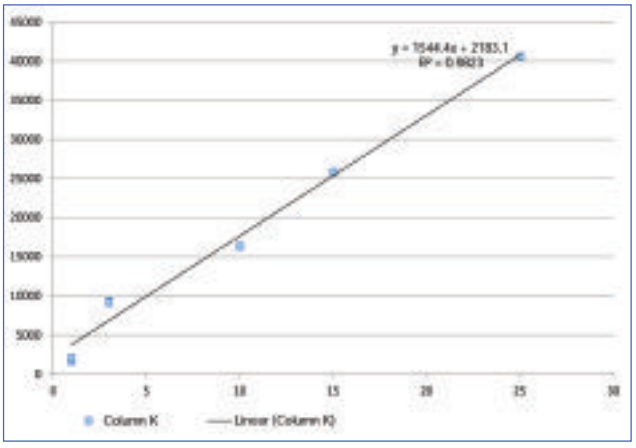


Figure 4. A calibration curve relating side scatter intensity to particle size.

Next we make measurements of prepared pitch samples from the paper machine of interest, and another of a model pitch sample. In the case of the model pitch sample this is an emulsion of olive oil, rich in triglycerides, and other tree derived fatty substances. *Figure 5* shows stickies (left) and model pitch (olive oil) on the right. The region of red coloured dots was used to create a gate, or a filter, for estimating the number of hydrophobic colloidal particulates present in a given sample. In excess of 90% of the particle populations in the above samples were present in the indicated red areas. With this information we can go ahead and make useful observations of real mill samples.

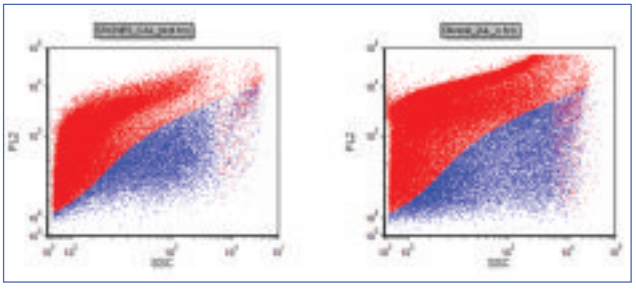


Figure 5. Fluorescence measurements on paper machine samples (left) and model samples (right).

Potential uses in Paper Making

This technique is already making in-roads into various industries, including brewing, food production, horticulture, water treatment, and bio-technology to name a few. The paper industry likewise is finding applications, and one simple example that I would like to discuss is that of surveying mill water systems. Very often mill technical and operational staff have an inherent hunch about where dirt and undesirable components accumulate in the complex mill water recycling systems. With flow cytometry it is possible to survey those systems, and gain insights into the qualitative nature of the water loop. For example, *Figure 6*

shows some measurements from a recycled packaging board mill in the UK:

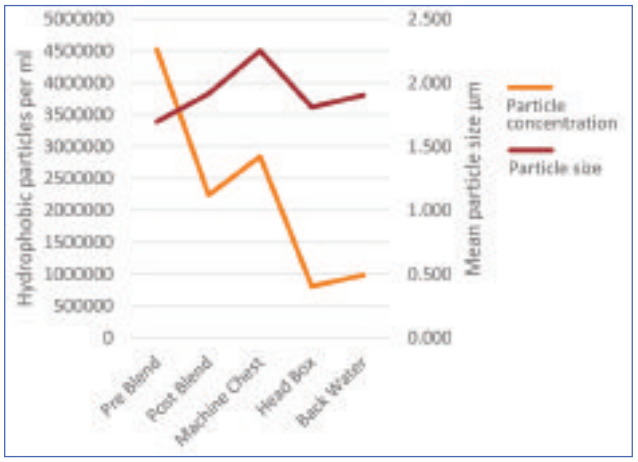


Figure 6. Chart of gated particle measurements across a packaging board mill in the UK. (Note: this chart is constructed from gated data, i.e. only counting colloidal material with a significantly hydrophobic nature, and as such can be regarded as process interference.)

This mill implements a chemical treatment in the form of a polymer based fixative, between the pre-blend and post-blend (chest) sample points, and as can be seen there is a 50% reduction in the gated population. Further along the process there is a slight rise in the colloidal hydrophobic population at the machine chest, possibly a consequence of the shear forces generated by the chest agitators. Still further along, at the head box, the full retention treatment has occurred, in this case a bentonite and cationic polymer system which gave rise to a further 50% drop in colloidal hydrophobic material as a direct result of retention. If you examine the size data at all points, the apparent mean size is bounded between 1.5 and 2.5 microns, and as such seem to be acceptable. If at some stage the mean particle size of a given location should rise abnormally and the process suffers repeated sticky/pitch deposition events, then you would need to consider what has changed to cause this disturbance and how to prevent apparent agglomeration of hydrophobic particles in the water phase. The two are highly probably connected. Overall the chemical treatments of the above mill seem to be performing well, and information like this can be used to create a baseline picture prior to any changes in process chemistry or engineering, and provide a valuable insight into the performance of mill systems, both before and after any such modifications. Additionally, by performing regular surveys of such a system any changes in the qualitative picture can be correlated with operational changes, and any negative effects can be closely monitored.

The next example concerns selection of an alternative chemical treatment. This case, I hope, will illustrate the benefits of flow cytometry to both the chemical supplier and the client. The relative benefits of two products can be quite subtle, and take many months of normal trending of machine data for the benefits to become apparent. This time I will compare the relative performance of various liquid polymer products, and aim to show that flow cytometry was able to give additional insight into selecting the correct product for an application. Prior to any decision about product choice a number of untreated process samples were taken and treatment simulations were performed in the laboratory to compare the various alternate products against the incumbent. Initially, process samples of untreated thin stock were taken, and flow cytometry analysis was performed with a view to understanding the nature and extent of the particulates in the water phase.

From the cytographs in *Figure 7* I created a couple of regions of interest that I hoped to find a suitable treatment to minimise any negative effects of moieties in these regions. The plots of side scatter (SSC) versus fluorescence (FL2) show the distribution of particulates based on size (SSC) in relation to their hydrophobic content (FL2). The plot of FL2 versus FL3 is a dual fluorescence plot, and it exhibits tighter population bunching, which in turn allows for easier segmentation of populations with similar properties. Observe the shape and distribution of the various gates created as illustrated by the dot plots of size versus hydrophobicity (SSC versus FL2). The purple gate is that of a hydrophobic gate derived from a model pitch sample, the red one was created to encircle the high populace region in the upper right section of the size versus fluorescence plot. The other gate

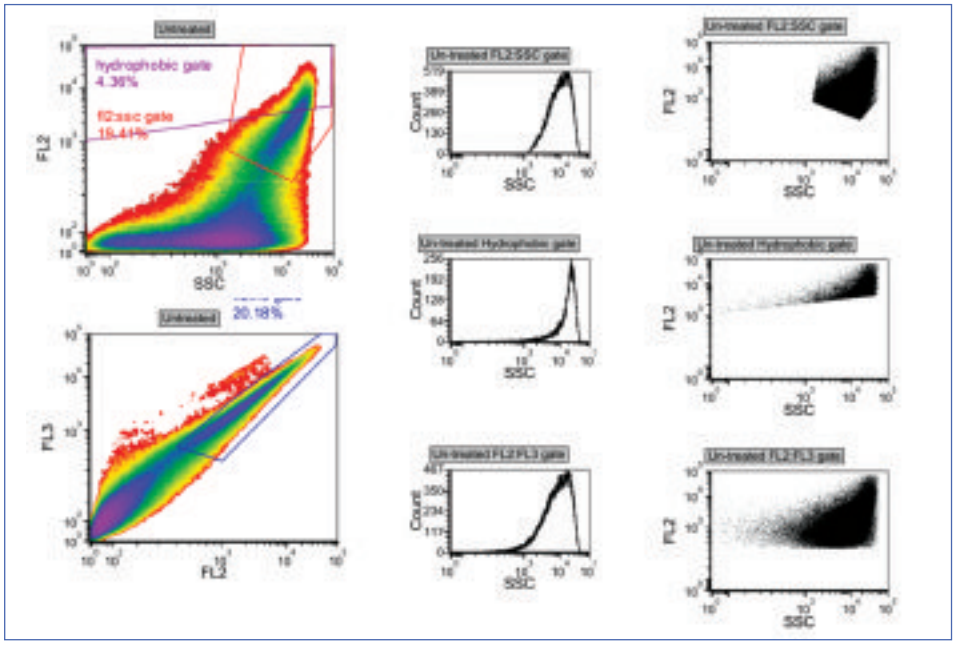
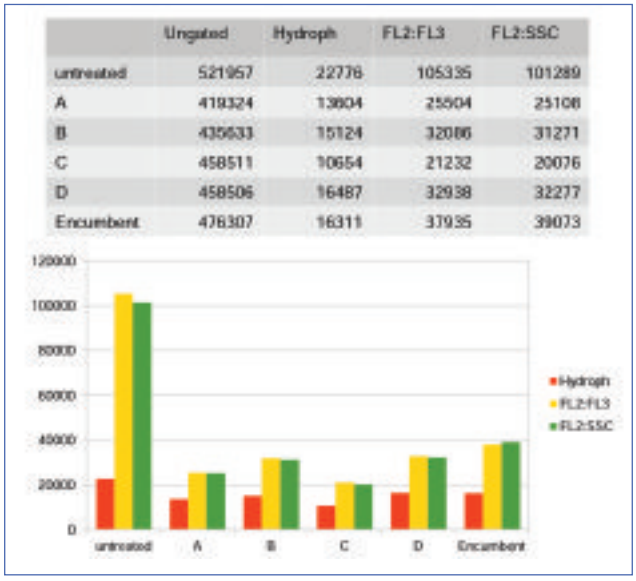


Figure 7. Cytograms taken from paper mill system.



	Ungated	Hydroph	FL2:FL3	FL2:SSC
untreated	521957	22776	105335	101289
A	419324	13604	25504	25106
B	435633	15124	32086	31271
C	458511	10654	21232	20076
D	458506	16487	32938	32277
Encumbent	476307	16311	37935	39073

Figure 8. Chart comparing effectiveness of various liquid polymer treatments.

coloured blue, was created from the dual fluorescence view, and as can be seen from dot plot has a more natural looking spread when viewed on the accompanying dot plot. For completeness I have included the more usual 1-D histogram of size versus count (SSC versus count). It is beyond the scope of this article, but let it suffice to mention that interaction between pitch and mineral particulates (bentonite, GCC etc.) is well known, and the effect is to quench the apparent fluorescence of the pitch, and increase the fluorescence of the mineral particulates. This effect is observed as regions of particulates at intensities below that of pure pitch. It is quite likely that the fluorescence observed in the middle of the range could well be a consequence of such interactions.[8]

With the fresh knowledge of the background particulates a series of dosage trials were performed at lab scale, and the resulting measurements collated on a spread sheet for comparison.

It should be immediately obvious that from the data in *Figure 8*, the optimal liquid polymer selection would be product C, when you consider particulate fixation alone in isolation. Naturally other factors would need to be considered, but as an additional metric it can be a very useful tool to have at hand.

Summary

One of my unstated aims was to make what can be a difficult technique to grasp, more accessible to a lay reader with little or no experience or knowledge of what flow cytometry is, let alone its potential and application in the industry we work in. As a method to support troubleshooting, and an aid to the chemical supplier, it is in my opinion becoming very useful indeed. There are caveats, in that populations or regions of interest cannot be identified in the same way an analytical chemist will identify all the chemical components in a provided sample. However valuable clues about the nature of the populations can be deduced, and their potential impact on paper making operations can be inferred. We (Axchem UK) will continue to investigate, and refine our knowledge and understanding of what is a quite interesting laboratory technique, that will no doubt become more important as paper makers seek to make water savings, and the composition of paper machine water loops begin to have more of an impact on the operational efficiency of paper mills.

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Other suggested reading:

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