The request for a urine status in the laboratory routine mostly concerns incoming orders within the scope of health checkups or screening tests, or problems relating to an existing urinary tract infection or possible haematuria. In the latter cases, the patients generally describe typical symptoms to the treating physician so that urinalysis is consequently requested from the laboratory to additionally confirm or exclude the possible diagnosis.

After urine flow cytometry had made its arrival in urinalysis more than 10 years ago, Sysmex today offers the fluorescence flow cytometers UF-1000i and UF-500i with the advantageous semiconductor laser technology for the analysis of urine. These systems have been further developed especially in terms of the most frequent findings in urinalysis; and now, the systems excel in the following:

- reliably selecting out normal samples
- more sensitive detection of samples with indications of urinary tract infections
- better detection of haematurias

Without any further preparations to be made, the tubes with the native urine can be placed on the sampler of the UF-1000i or UF-500i analyser directly after having opened them. The urine sample will be automatically mixed, and a volume of 1,200 μL will be aspirated for analysis (Figure 1).
In the UF-1000i and UF-500i analysers, the urine sample is split into two separate aliquots and mixed with special reagents at a fixed dilution ratio. The sample aliquots will thus be prepared for the subsequent analysis processes. The following addition of polymethine dyes – one for the bacteria counting, the other for analysis of the additional particles – will then show optimum staining because specific pH values have been adjusted by the respective diluting reagents: With the addition of the diluents, the membranes of the cells to be stained will be pre-treated so that the staining of the desired cell components with the polymethine dyes will be facilitated. Staining takes place in incubation chambers adjusted to specific temperatures, optimising the process for both aliquots.

By using polymethines in the UF analysers, a number of advantageous properties of these dyes will be exploited at the same time. Polymethines can be synthetically produced. With the number of methine groups as well as the number and type of branchings, the absorption spectrum of polymethines can be changed and thus easily adjusted precisely to the wavelength of the laser used in the UF instruments which, at 635 nm, is in the range of the red light. Polymethines are compounds consisting of \( x \) atoms \((x = 2n+1; n = 1, 2, 3 \ldots)\) and \( x-2 \) methine groups, and whose \( x \) molecule orbitals are occupied in pairs with \((x+1)\) \( \pi \) electrons. These \( \pi \) electrons are completely delocalised and function as highly sensitive photo receptors which absorb the energy-rich laser light and subsequently emit fluorescence light (wavelength > 660 nm, near infrared range) when the diluted urine with its stained particles passes the laser beam in the flow cell (Figure 2).

**Fig. 2** Optical unit of the UF-series analysers
Hydrodynamic focusing through a sheath flow reduces the diameter of the diluted and stained urine stream upon entry into the flow cell. The particles thus pass the laser beam individually and aligned in length, at a high velocity. For each cell, forward scattered light and – with today’s UF models – also side scattered light will be simultaneously registered. Laser-induced fluorescence light will also be registered. Optical signals and thus the characteristics of up to 65,000 particles – as far as present in the analysis volume of the urine – will be analysed and are used for the classification in common urinary particles and the generation of additional analytical information.

The analysis results will be presented on the display after barely 1.5 minutes and offer further improved quality in terms of the typical urinalysis issues stated in the beginning:

**Reliably selecting out normal samples**

Normal samples are detected with the Sysmex UF-series at high sensitivity, also contributing to a high negative predictive value. Especially for screening programs with normal patients, the highest possible sensitivity is required to recognise as reliably as possible patients having potential diseases. The high negative predictive value, on the other hand, gives confidence in the negative results indicating that disease is actually absent.

High sensitivity is reached, inter alia, by
- classifying a great number of particles;
- analysing native urine to circumvent, from the start, the known sources of error in sediment microscopy;
- staining by using specifically developed dyes under optimised conditions.

The results of negative samples – as a rule representing the major part of the urine samples received in the lab – can be immediately automatically validated, transmitted to the LIS, and reported to the requesting physicians.

**More sensitive detection of samples with indications of urinary tract infection**

The detection of possible urinary tract infections is one of those examinations for which the treating physician wants to receive fast feedback. Possible urinary tract infections should, of course, not be overlooked; any samples analysed as negative should actually be negative, as far as is possible; and particularly urine samples coming from specific patient groups, such as, for example, those from intensive care units, and showing indications of a possible urinary tract infection should not be overlooked.
With the development of a reagent system which exclusively stains bacteria and does not register cell debris, the UF-1000i and UF-500i are now able to better determine bacterial values, even in the clinically relevant range of concentrations between $10^3$/mL and $10^4$/mL. Combined with the simultaneous detection of white blood cells and yeasts, the treating physician can be immediately informed as to whether one or more of these three infection indicators could be detected. In the clinical environment, fast reporting of negative results can contribute to reduce blindly started antibiotics therapies – to thus reduce costs and prevent resistances.

**Better detection of haematurias**

The detection of samples with indications of possible haematuria also requires high analytical sensitivity since such findings must generally be further clarified because the possible source of bleeding and its cause must be found.

In the detection of haematurias, special attention has been attributed to an improved specificity without reducing the sensitivity. The number of false-positive red blood cell values (RBC) could be minimised with the analysis of the side scattered light since possible interferences between crystals and red cells were significantly reduced. Due to their numerous forms and surface structures, crystals provide typical side scattered light signals and can thus be well differentiated from red blood cells (Figure 3).

*Fig. 3* The different forms and surface structures of crystals and red blood cells develop typical side scattered light signals.
In the end, the quality of red blood cell detection could thus be improved on the UF-1000i and UF-500i which, of course, positively influences the additional RBC information. When haematuria is now detected the automatic, initial categorisation, e.g. into ‘dysmorphic RBC?’ (referring to the possible presence of dysmorphic red cells) or ‘isomorphic RBC?’ (referring to red cells of normal size/shape), is based on an improved red cell analysis which then should induce further examination if ‘dysmorphic RBC?’ was indicated.

Hardware and software as well as the reagent system implemented in the UF-1000i and UF-500i are brilliant in their easy handling and operation. Their attractive design and the truly convincing and appealing software of the IPU (information-processing unit) will enable fast familiarisation with the operation of the UF-series analysers which render modern urinalysis clearly more attractive (Figure 4).