INTRODUCTION

The quantitative characterization of the dispersed phase size distribution in a biphasic system is a problem of both scientific and industrial interest for a variety of materials, including emulsions, polymer blends and suspensions. In order to get statistically significant results, a large number of inclusions need to be identified. To characterize the size distribution, however, only a limited number of particles is usually measured, and the statistics, as calculated on this subsample, is not always representative of the whole population.

In fact, it has been shown that in particle systems it is possible to estimate the random sampling error, a measure of how much a subsample is representative of the whole population, only provided the sample size is large enough, i.e. the number of inclusions measured is larger than a critical value, which depends on the size distribution [1-2]. The number of measurements needed is often of the order of several thousands, in order to limit this error to less than 10%.

A similar task can only be handled by automated methods. Image analysis, however, is a non-trivial problem even in the case of semidilute biphasic systems, due to the presence of out-of-focus components. In particular, the image quality decreases by focusing more deeply inside the sample, due to the presence of inclusions in the adjacent layers along the optical path. The image degradation depends on the volume fraction and size of the inclusions, and on the relative refractive indices of the two phases. When the latter are rather high, the quality of the images can be severely affected by lensing effects [3]; in fact a drop may act as a spherical lens, generating distorted images of other drops located in lower focal planes. This effect can be observed in some of the drops shown in Figure 2a. On the other hand when the refractive indices of the two phases are very close to each other [4] the recognition of the dispersive phase is non-trivial due to the difficulty in identifying the border of the inclusion.

We propose here a new technique to automatically detect spherical inclusions in a biphasic system. The particles are measured and located in the sample, allowing a statistically significant evaluation of the size distribution and 3D positioning of the inclusions. We describe several examples of the application of this technique to different samples, such as solid, liquid and gas inclusions in liquids, in the 5-80 micrometre size range. Samples with volume fractions of the dispersed phase up to 15% have been analysed.

MEASURING TECHNIQUE

This tool is a direct answer to the problem of characterising the particle size distribution in a non-dilute biphasic system through image analysis. The proposed technique is based on 3D optical sectioning of the sample with a light microscope. Images are digitised for off-line analysis. The technique is based on three subsequent steps: 1. Image acquisition; 2. Image analysis; 3. Data processing and statistics.

IMAGE ACQUISITION

In our experimental setup, observations have been performed in transmitted light, using a Zeiss Axiotop SF with a Hitachi KP-ME1 black and white CCD camera. The optical observations were done using a 20×, NA 0.40, Zeiss Achroplan long working distance objective with a depth of field of 3.23 µm [5] and a 100×, NA 1.25, Zeiss Achromplan oil immersion objective with a depth of field of 0.41 µm [5]. The sample was optically sectioned by exploiting the focus drive of the microscope; images of several layers taken at different depths in the sample were acquired by changing the microscope focus. The distance between consecutive layers was set below the depth of field of the lens used. The procedure can be iterated to acquire several of such image stacks by translating the sample in order to scan a large portion of the sample through a 3D motorised microscope. To this end, the sample was put on a x-y motorised translating stage and the focus was controlled by a stepper motor directly connected to the coarse gear of the microscope. Ad hoc software routines have been implemented to perform sample scanning and image acquisition. Images were digitised by a framegrabber and stored on the hard drive for off-line analysis.

IMAGE ANALYSIS

Image analysis algorithms have been developed to automatically process the large number of images acquired. The images are grouped in different stacks from the scanning procedure, and each stack is independently analysed.

The basic steps of the algorithms are out-of-focus removal, image segmentation, edge detection, and identification of the best focus layer of each inclusion. The inclusions are assumed to be spherical, such being the shape of a dispersed phase droplet at equilibrium. Every inclusion is then defined by its x, y, and z position and its radius.

Due to the turbidity of the mixtures, an increasing image degradation was noticed when focusing inside the sample, depending on the size and number of the inclusion, i.e. the volume fraction of the dispersed phase and its size distribution. Significant differences...
in the quality of the images were also related to the difference in the refractive indices of the two phases. When the refractive index of the dispersed phase is sensibly different from that of the matrix phase, the presence of inclusions in the adjacent layers generate lens(ing) effects that can distort the image of the in-focus inclusions, depending on the size and curvature of the out-of-focus inclusions [3]. In the case of spherical inclusions, e.g. drops or bubbles, the final distortion effect resulted in a smaller apparent size of the inclusion and in a biased focus position. Overall, these distortions resulted in a false peak in the small size region of the size distribution. This problem was corrected by developing post-processing algorithms taking into account the presence of neighbours around each inclusion.

In the following, we report some examples of application of the image analysis algorithms on different biphasic systems (liquid-liquid, gas-liquid and solid-liquid) characterised by different optical and physical properties. We present both the raw images and the results of the image analysis algorithms.

A first example of image processing is shown in Figure 1. The sample is a three component mixture of water, gelatin and dextran that separates into two immiscible phases.

In Figure 1a, a raw image of a 3% wt mixture of the two immiscible phases is shown. The refractive indices of the two phases have been measured with an Abbe refractometer and were 1.357 for the dispersed phase liquid and 1.365 for the continuous phase liquid.

In Figure 1b drop contours, as identified by image processing (red circles), are overwritten for comparison. It can be noticed that most of the drops which appear in focus in the raw image are well detected by image processing.

In Figure 2a an image of a 10% mixture of polydimethylsiloxane (PDMS) in polyisobutylene (PIB) is shown. Both polymers are immiscible and liquid at room temperature with refractive indices of 1.403 (PDMS) and 1.495 (PIB). The lensing effects due to the presence of drops in layers above the one in focus are evident.

In Figure 2b the result of the analysis of this image is presented. Most of the distortions due to lensing effects have been taken care of, thanks to the correction algorithms previously mentioned.

In Figure 3 a raw and an analysed image of air bubbles in water are shown and similar conclusions can be drawn. In this case the limited size of the bubbles needed an higher magnification (100× oil immersion).

In Figure 4 the analysis is applied to a solid-liquid system. The dispersed phase is made of polystyrene spheres suspended in immersion oil (Zeiss, refractive index 1.518). Also in this case, the image in Figure 4b reports the result of the analysis.

**DATA PROCESSING AND STATISTICS**

After having processed all the acquired images, the results of the analysis were stored in files. For each inclusion the radius, the x-y position and the best focusing layer as chosen or measured by the image analysis algorithms, were recorded. Image analysis to collect these data, although completely automated, was the most time-consuming step of the whole process: about 10 s per image was needed on a Pentium IV 2.8 GHz computer, depending on the number of objects present in the image and on the image quality. These data were then stored to be post-processed to generate the observable outputs.

First of all, the statistical methods described before [1,2] were applied to verify the consistency and reliability of the measured data. Briefly, the random sampling error was calculated according to the theoretical predictions taking into account the measured size distribution of the sample and the number of inclusions measured. In this way it was possible, for example, to have an estimate of the error associated with the mean or the variance of the size distribution. As mentioned in the introduction, in order to limit this error to less than 10%, the sample size, i.e. the number of particles to be measured, was often on the order of several thousand, depending on the variance of the distribution. It must be noted that the estimate of the random sampling error is valid only if the sample size is bigger than a
critical value. If this is not the case, the error can be significantly underestimated. Details of the calculations are beyond the scope of this article but can be found in references [1,3,4].

The proposed technique is completely automated, allowing the user to identify a number of particles large enough to measure not only moments like average and variance with statistical significance, but also to get a good representation of the whole size distribution of the dispersed phase.

The optical sectioning procedure allows one also to detect the exact position of the inclusions inside the sample. In particular, it is possible to obtain a 3D reconstruction of the inclusions detected in each stack of the sample.

Figure 5 shows a typical rendering which is the result of the analysis of a 5% wt mixture of the two water-based immiscible fluids shown in Figure 1. This type of analysis can be used, for instance, to assess emulsion stability, by monitoring drop sedimentation or system heterogeneity [3,4] as a function of time, both under static conditions or during flow. In the latter case, wall effects can drive the drops to concentrate in the centre of a channel or a pipe, and this phenomenon can be quantified by this analysis.

**PERFORMANCE**

The performance of the image analysis procedure has been thoroughly tested by comparison with manual analysis of selected stacks of images from different experiments. Manual analysis was performed by saving all the images of a stack in a movie format (such as AVI), which can be played to facilitate the location of the best focus position of each drop, and by overlapping a circle to the drop contour through interactive measuring tools provided by most commercial software packages. The radius of each superimposed circle and the co-ordinates of its centre were then recorded on file for statistical analysis.

An example of the comparison is presented in Figure 6. The bottom graph shows the distribution of drop size corresponding to two stacks of images containing around 500 drops; the two curves correspond to the automated measure and to the manual analysis. In the top graph the cumulative count of the two distributions is plotted. It can be seen that automated and manual analysis are in good agreement with each other. In particular, this can be quantified by directly comparing the values of the first two moments of the distribution, i.e. average size and variance, as calculated from automated and manual analysis. We found that the discrepancy between the two values was generally below 5%.

Concerning the drop size range that can be explored with our technique, a lower limit is imposed by optical resolution, which depends in turn on the numerical aperture of the objective used. Further limitations are introduced by digitisation of the video signal and image degradation due to sample turbidity. Taking into account all these effects, we estimate that in our experimental conditions the lowest drop size that can be analysed with confidence is around 0.8 µm.

**CONCLUSIONS**

We have presented here a new methodology to characterise particle size distribution in two-phase systems. The technique is based on 3D optical sectioning of the sample through motorised microscope control and off-line image analysis. The particle size distribution is determined and statistical methods are used to estimate the sampling error. A 3D reconstruction of the microstructure is also performed, thus allowing one to estimate possible sample heterogeneity. The method has been widely applied and tested on systems with different characteristics and properties and showed to be versatile and reliable.

**REFERENCES**