



**FIGURE 19-5** Merging of results from two different hybridizations of the same microscope slide. In the first hybridization (a), the *ABL* gene was stained with FITC (green), the *BCR* gene was stained with rhodamine (red), the counterstain was DAPI (blue), and the image was acquired in wide-field mode. In the second hybridization (b), the territory of chromosome 9 was stained with rhodamine (red), the territory of chromosome 22 was stained with FITC (green), the counterstain was DAPI (blue), and the image was acquired in confocal mode. Only a part of the whole field of view is shown (about  $60\ \mu\text{m} \times 45\ \mu\text{m}$ ) for both hybridizations in so-called autofocus (maximum-projection) mode. Each hybridization was analyzed separately. The results of the analysis are visualized using small circles for genes (c) and territory highlighting for chromosomes (d). The visualization color was set identical to the original emission color of the genetic loci. The nuclear boundaries (closed curves) and weight centers (crosses) are highlighted in cyan (first hybridization) and magenta (second hybridization). The two independent analysis results were subsequently overlaid (e), and corresponding pairs of nuclei were determined. Because of microscope-stage imprecision, the positions of nuclear weight centers do not match—they are shifted by a certain vector. After the determination of this vector, the nuclei can be correctly overlaid (f), enabling visualization of the gene positions within chromosome territories. Reprinted from Kozubek M. et al. (2001).