CHAPTER 7

CONCLUDING OVERVIEW

In the work presented here, microfluidics has been used as an important tool to study reactions at the single-molecule level. It does not only provide an alternative approach for quantification via compartmentalization, but also has the capability to decouple different stages and facets of one reaction, which facilitates the study of reaction mechanisms. This is usually difficult for or beyond the capability of other technologies.

Microfluidics provides possibilities for performing molecular diagnosis in limited-resource settings. The confinement of single molecules converts analog signals to digital signals. Such conversion allows the readout to be carried out only at the endpoint of the reaction, rather than real-time monitoring of the entire reaction progress[16]. Furthermore, by confining molecules into different volumes in the same assay and incorporating mathematical considerations, the dynamic range can be enlarged to satisfy the requirements of viral load quantification[32]. In addition, isothermal amplification chemistry could be employed to eliminate the requirement of thermal cycling[35]. All the advances discussed above enable SlipChip microfluidics devices to be used in limited-resource settings for the detection and quantification in diagnostic assays.

The two most important characteristics of such a digital assay—efficiency and robustness—have been studied and a novel concept has been proposed to describe the progress of some reactions. When a reaction is observed at the single-molecule level, the probability for each molecule to initiate the reaction (fate) and how fast the reaction proceeds (rate) can be looked at separately, unlike in a bulk format. The decoupling of “fate” and “rate” in a digital format could increase the assay sensitivity, as in the example of HCV genotyping using RT-LAMP and restriction endonuclease. Furthermore, the monitoring of reaction progress for each single molecule can aid

understanding of whether the presence of any perturbation changes the “fate” or “rate” of a reaction.

The understanding of reaction mechanisms at the single-entity level enabled by microfluidics aids the design and development of new assays to solve real-world problems, which has been demonstrated with applications in HIV and HCV viral load quantification and HCV genotyping. It can also potentially address problems not currently solvable by existing technologies: for example, the co-incidence detection of multiple biomarkers, studying the heterogeneity of cells, bacteria, organisms and molecules, and so forth.

As we have stated in a review[8] by our group, the adoption of single-molecule microfluidics in biology and chemistry should also be accompanied by the effort of making these technologies massively distributed, in terms of both cost and ease of operation. The replacement of glass material by low-cost plastic material has been shown in my work[42] and is one of the future directions for microfluidic platforms to be widely used. The integration of many components for a final “sample-in-result-out” assay is also necessary. Most of the work discussed here focused on the amplification chemistry and detection reaction, but other questions, such as how to extract target nucleic acids from raw sample (such as blood, plasma, and swab) and to test their compatibility with downstream reactions, remain to be answered. Once these elements are tested and characterized, microfluidics will provide unique advantages and opportunities for biology and chemistry.