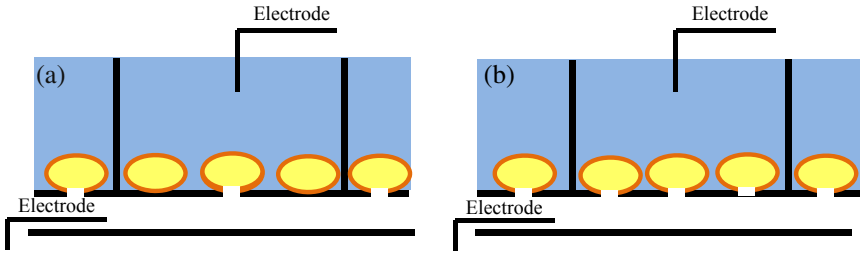

FIGURE 4.40 The development of 96 plates suitable for electrophysiological experiments has facilitated the identification of compounds capable of modulating ion channel activity. In the IonWorks™ PatchPlate system (a), each well of the plate has a single hole at the bottom that can be used to access the inside of a single cell for electrophysiological experiments on one cell per well. Population patch clamp (PPC) plates (b) have 64 holes at the bottom of each well and provide information on electrical changes across a small population of cells per plate well.

hole in each plate (Figure 4.40(a)). Application of a slight vacuum to the underside of the plate keeps the single cell in place, while the addition of the pore forming antibiotic amphotericin provides electrical access to the cell, thus permitting patch clamp experiments in a plate-based system. Automated liquid handlers built into the IonWorks™ HT platform further streamline the process.[87]

In theory, moving from single compound screening to a 384 well plate-based system should provide a 384-fold increase in screening capacity, but in practice the increase in throughput is much lower. The electrical seals established in perforated patch clamp method have substantially lower electrical resistance than manual methods. This leads to well to well variability, which is further complicated by a relatively high failure of cells to be properly placed over the holes at the bottom of the well. These limitations necessitate multiple wells per assay condition in order to provide useful data.

The introduction of population patch clamp (PPC) mode of operation and the IonWorks™ Quattro system have overcome some of these limitations by not relying on a single cell recording per well. The PatchPlates employed in PPC mode have 64 holes per well and measure the electrical changes as a population average measurement (Figure 4.40(b)). Although this does not address the issue of lower electrical resistance seals than that available via manual methods, it does decrease the failure rate by decreasing the likelihood that cells are not properly positioned within the microtiter plate. Irrespective of this limitation, however, the ability to use plate-based technology for patch clamp determination represents a significant advance in capacity over manual methods.[88]