Figure 2.3: The semi-automatic data analysis pipeline for mitotic duration assays.

The phase-contrast image shown here is not a real image (but the exemplary kernel is one of the kernels that were used in this study). Real phase-contrast images are complicated by nearby or underlying interphase/prophase cells, dead cells that might also have a similar halo, and irregularity in the shape and size of the mitotic cells. Large displacement of a mitotic cell and local crowdedness may also be problematic to automatic tracking. Therefore, manual examination and correction of all identified mitotic events are necessary.
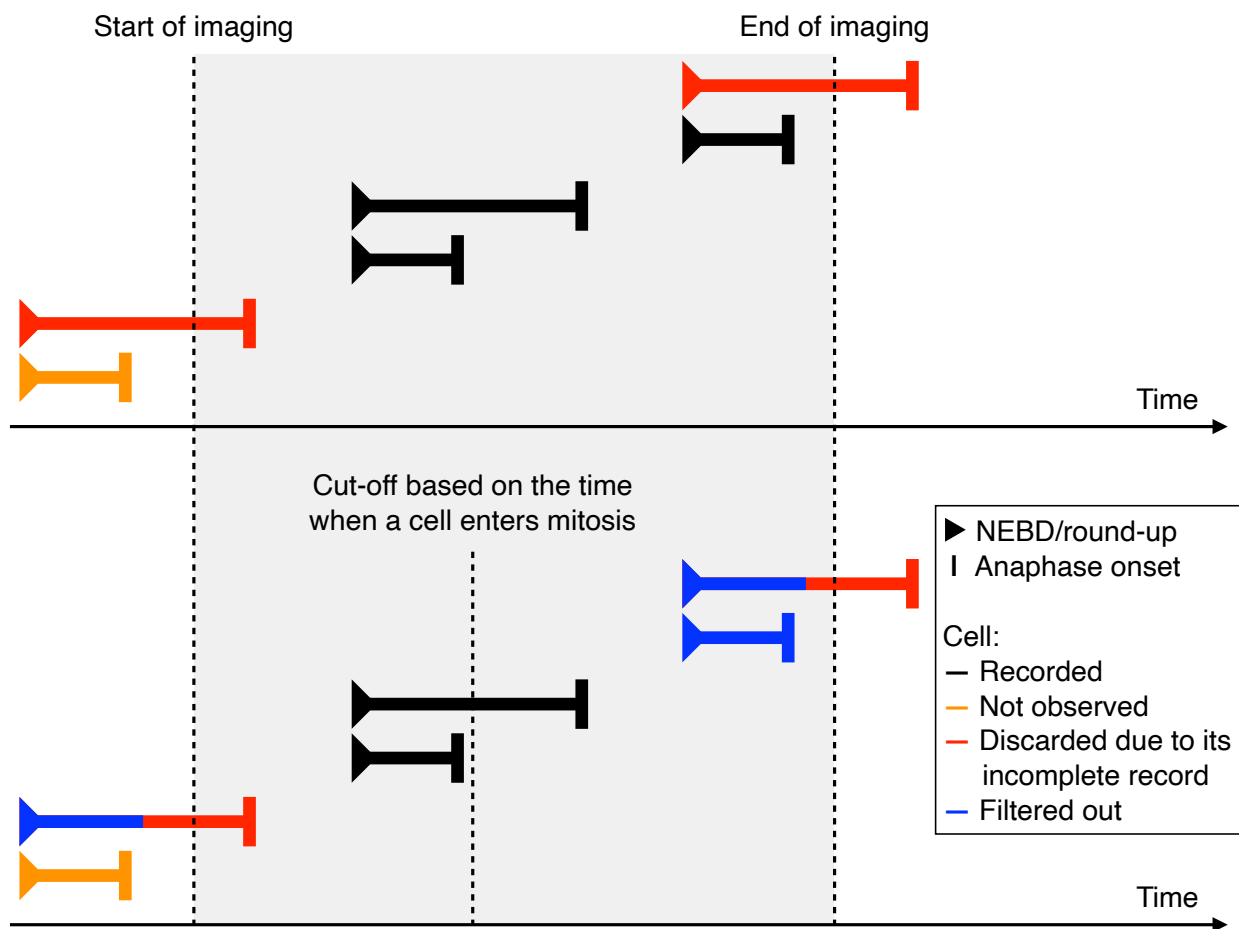
Figure 2.4: The observer bias in the data analysis of the mitotic duration assay can be eliminated by extending the duration of the time-lapse imaging and setting a cut-off line to filter out cells whose NEBD timing is too late.

In this simple example, we have two types of cells. One of them arrests in mitosis for a short duration while the other arrests in mitosis for longer. The ratio between the total numbers of the two types of cells is 1 : 1 and the NEBD timing is independent of the duration from the NEBD to the onset of anaphase. If no anti-bias measure is taken (as illustrated in the top panel), the observed ratio between the two will be distorted (2 : 1). However, if we set a cut-off line to filter out cells whose NEBD timing is too late (as illustrated in the bottom panel), the observed ratio will be restored to 1 : 1, faithful to the true ratio. As long as the margin from the cut-off line to the end of imaging is longer than the mitotic duration of most cells, this bias should be mostly eliminated.