Protocol: Cell Splits Analysis Example: Inclusion Bodies

Because nearly all processes during the cell-cycle are unsynchronized from neighboring cells, e.g. cell length, cell-cycle time, chromosome replication and segregation, etc., a single field of view may contain cells in nearly all stages of the cell-cycle. This, coupled with the fact that we follow cells through multiple division events, allows us to characterize single cell protein localization as a function of many non-trivial cellular characteristics, such as cell length, cell-cycle length, cell birth time, number of cell neighbors, pole age, and total fluorescence, that are simply not possible in snapshot analysis.

For example, it has long been reported that excess protein in the cell tends to aggregate at the cell poles into *inclusion bodies.* But, each cell contains different 'aged' poles, i.e. one pole came from a division event (new-pole) and one did not (old-pole). If inclusion bodies do indeed collect at the poles, we would expect to find that older cell poles on average contain more inclusion bodies than younger poles. In order to investigate, we find the mean pole age and split our single cell data cells into groups on either side of the mean. The consensus and splits data is shown below for an example strain (*carB*). The split data clearly shows two distinct populations of polar localization as a function of pole age: cells with older poles tend to contain more polar-localized fluorescence, consistent with the hypothesis of inclusion body aggregates.

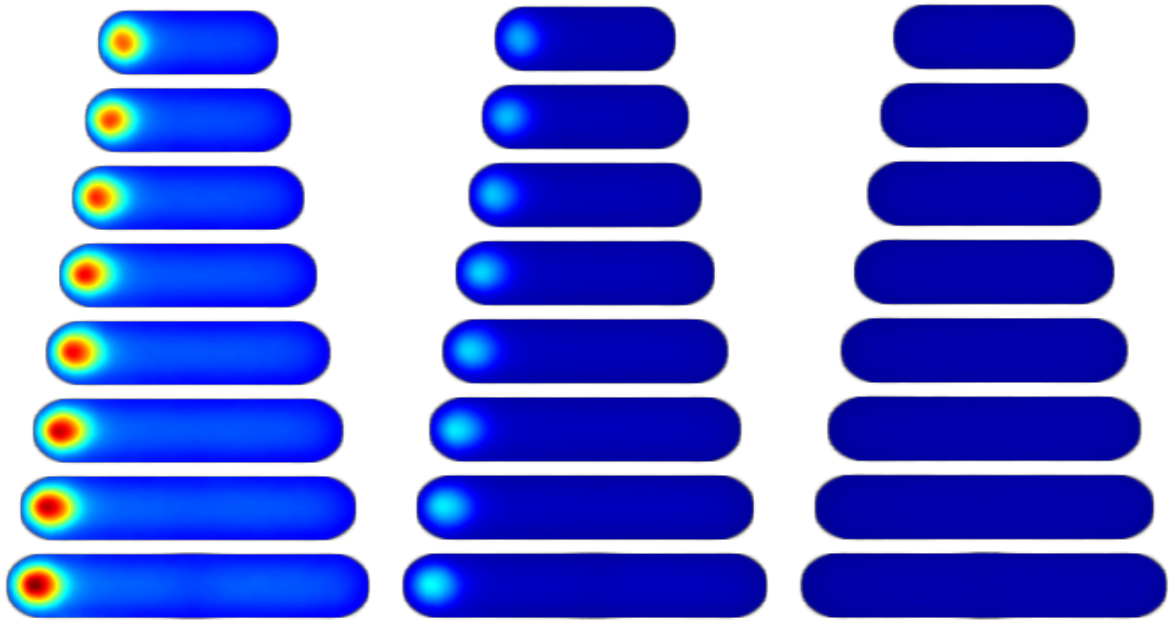

Figure 1: Polar localization as a function of pole age. Cells with older poles tend to have mor polar-localized fluorescence that cells with younger poles, consistent with the hypothesis of inclusion body aggregation at the cell pole.