

**Supplementary Note 1**

| <b>Main Text Figure</b>  | <b>Cell Type</b>                         | <b>Optical Modality</b>                      | <b>Objective Magnification</b> | <b>Objective Numerical Aperture</b> | <b>Camera</b>                 |
|--------------------------|------------------------------------------|----------------------------------------------|--------------------------------|-------------------------------------|-------------------------------|
| Figure 2a                | MDA-MB-231 (human breast adenocarcinoma) | Phase                                        | 10X                            | 0.3 (air)                           | Zeiss AxioCam 702 CMOS        |
| Figure 2b                | MDA-MB-231                               | Bright-field                                 | 40X                            | oil                                 |                               |
| Figure 2c.i              | MDA-MB-231                               | DIC                                          | 20X                            | 0.8 (air)                           | Zeiss AxioCam 702 CMOS        |
| Figure 2c.ii, 9a         | <i>S. cerevisiae</i>                     | DIC                                          | 63X                            | 1.4 oil                             | Zeiss AxioCam 702 CMOS        |
| Figure 2d                | Hs27 (human foreskin, fibroblast)        | IRM                                          | 40X                            | 1.4 (oil)                           | Hamamatsu ORCA R2 CCD         |
| Figure 2e.i,ii           | A549 (human lung adenocarcinoma)         | Epifluorescence (GFP)                        | 100X                           | 1.46 oil                            | Hamamatsu ORCA R2 CCD         |
| Figure 2e.iii,iv         | MDA-MB-231                               | Epifluorescence (F-actin and Vinculin)       | 63X                            | 1.25 (oil)                          | Photometrics CoolSNAP HQ2 CCD |
| Figure 3a                | Hs27                                     | Epifluorescence (DAPI, F-actin and Vinculin) | 20X                            | 0.8 (air)                           | Zeiss AxioCam 702 CMOS        |
| Figure 3b                | Hs27 (human foreskin, fibroblast)        | Confocal Fluorescence (F-actin and Vinculin) | 63X                            |                                     |                               |
| Figure 4                 | Hs27                                     | Epifluorescence (Zeiss3) (Vinculin)          | 40X                            | 1.4 (oil)                           | Hamamatsu ORCA R2 CCD         |
| Figure 5, 6a – c (iv-vi) | Hs27                                     | Confocal Fluorescence (F-actin)              | 63X                            |                                     |                               |
| Figure 6a – c (i-iii)    | Hs27                                     | Epifluorescence (F-actin)                    | 10X                            | 0.3 (air)                           | Zeiss AxioCam 702 CMOS        |
| Figure 6a – c (iv-vi)    | Hs27                                     |                                              |                                |                                     |                               |
| Figure 8a-b              | Hs27                                     | Epifluorescence (Vinculin)                   | 10X                            | 0.3 (air)                           | Zeiss AxioCam 702 CMOS        |

**Supplementary Note 2:** Self-supervised learning segmentation approach before and after applying declumping step

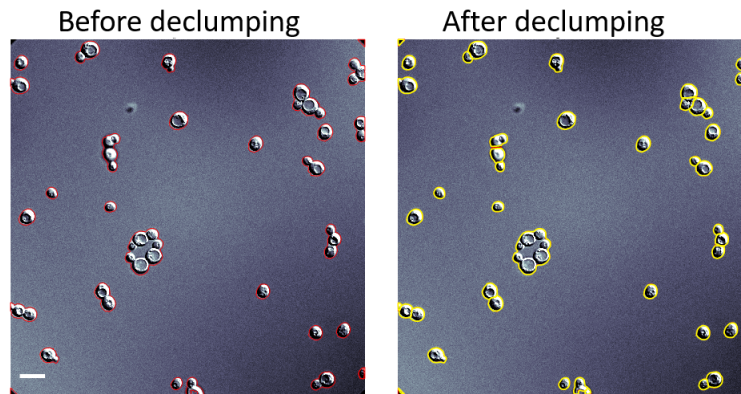


Figure: Self-supervised learning segmentation approach on *S. cerevisiae* image taken by DIC (63X objective) with and without declumping step. Scale bar: 5 $\mu$ m

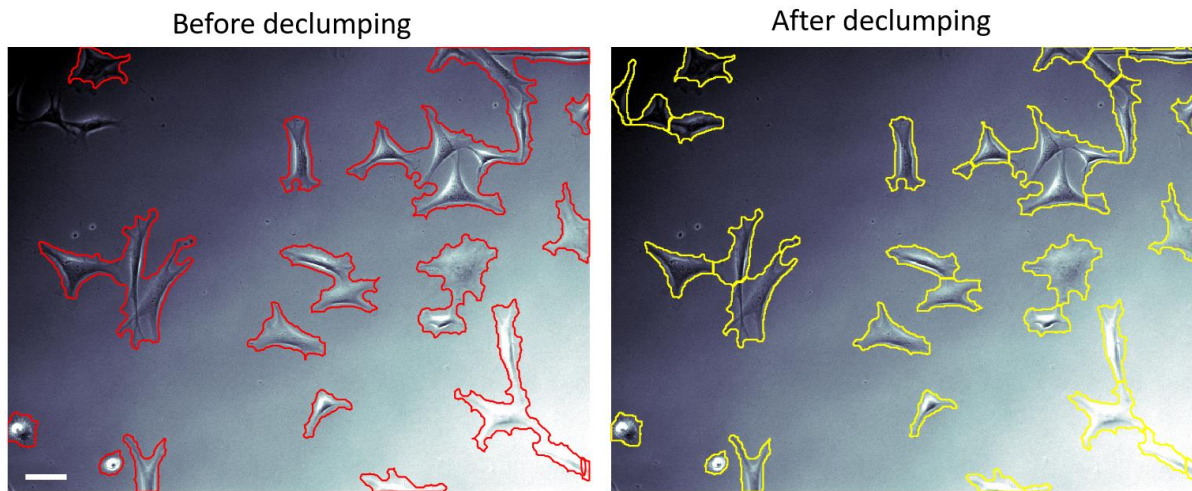


Figure: Self-supervised learning segmentation approach on Hs27 image taken by phase microscopy (10X objective) with and without declumping step. Scale bar: 50 $\mu$ m

**Supplemental Note 3: Summary of segmentation performance by SSL and different Cellpose models on several imaging datasets.**

|                                                          | Model                  | % of exported segmentation images | F1 score     | Time (minutes) |
|----------------------------------------------------------|------------------------|-----------------------------------|--------------|----------------|
| <b>Epifluorescence Hs27 60X</b><br>Number of images = 67 | SSL                    | 100%                              | <b>0.758</b> | 19.03          |
|                                                          | cp cyto (d=50)         | 82.09%                            | 0.078        | 12.07          |
|                                                          | cp_cyto (d=100)        | 49.25%                            | 0.119        | 8.33           |
|                                                          | cp_cyto (d=200)        | 38.81%                            | 0.192        | 9.47           |
|                                                          | cp_cyto (d=300)        | 29.85%                            | 0.253        | 8.11           |
|                                                          | cp_cyto (d=400)        | 35.82%                            | 0.339        | 13.73          |
|                                                          | cp_cyto2 (d=50)        | 100%                              | 0.059        | 12.54          |
|                                                          | cp_cyto2 (d=100)       | 85.07%                            | 0.195        | 10.58          |
|                                                          | cp_cyto2 (d=200)       | 82.09%                            | 0.455        | 15.64          |
|                                                          | cp_cyto2 (d=300)       | 68.66%                            | 0.674        | 26.83          |
|                                                          | cp_cyto2 (d=400)       | 62.69%                            | 0.608        | 33.80          |
|                                                          | cp_cyto3 (d=50)        | 86.57%                            | 0.072        | 14.78          |
|                                                          | cp_cyto3 (d=100)       | 70.15%                            | 0.191        | 17.21          |
|                                                          | cp_cyto3 (d=200)       | 74.63%                            | 0.337        | 36.2           |
|                                                          | cp_cyto3 (d=300)       | 67.16%                            | 0.400        | 43.95          |
|                                                          | cp_cyto3 (d=400)       | 53.73%                            | 0.454        | 38.63          |
| <b>Epifluorescent Hs27 10X</b><br>Number of images = 20  | SSL                    | 100%                              | 0.8877       | 10.49          |
|                                                          | cp_cyto2 (d = 50)      | 100%                              | 0.795        | 11.05          |
|                                                          | cp_cyto2 (d = 100)     | 100%                              | 0.882        | 16.67          |
|                                                          | cp_cyto2 (d = 150)     | 100%                              | 0.792        | 16.56          |
| <b>Phase Contrast Hs27 10X</b><br>Number of images = 565 | SSL                    | 100%                              | 0.771        | 129.33         |
|                                                          | cp_cyto2 (d = 30)      | 100%                              | 0.622        | 142.00         |
|                                                          | cp_cyto2 (d = 50)      | 100%                              | 0.777        | 148.05         |
|                                                          | cp_cyto2 (d = 100)     | 100%                              | 0.614        | 118.7          |
| <b>Epifluorescence Nucleus</b><br>Number of images = 189 | SSL                    | 100%                              | 0.873        | 23.47          |
|                                                          | cp_nuclei (d = 30)     | 100%                              | 0.953        | 58.01          |
|                                                          | SSL + cp_nuclei (d=30) | 100%                              | 0.944        | 76.03          |
|                                                          | SSL + declumping       | 100%                              | 0.873        | 24.22          |
| <b>Epifluorescence Hs27 40X</b><br>Number of images = 17 | SSL                    | 100%                              | 0.831        | 3.02           |
|                                                          | cp_cyto2 (d=150)       | 82.35%                            | 0.818        | 5.154          |
| <b>DIC S. cervia 63X</b><br>Number of images = 60        | SSL                    | 100%                              | N/A          | 36.35          |
|                                                          | yeast_PhC_cp3 (d= 50)  | 100%                              | N/A          | 65.67          |
|                                                          | SSL + declumping       | 100%                              | N/A          | 39.14          |