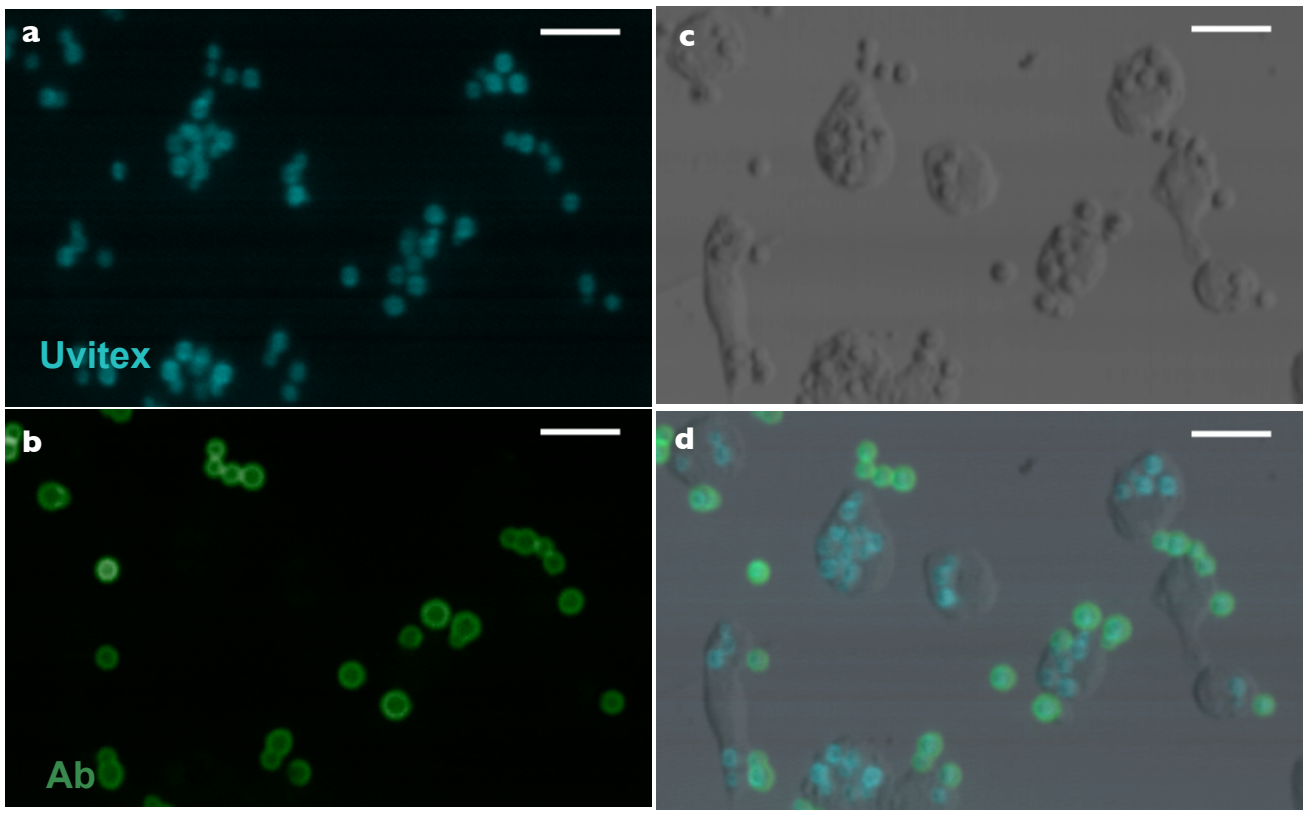
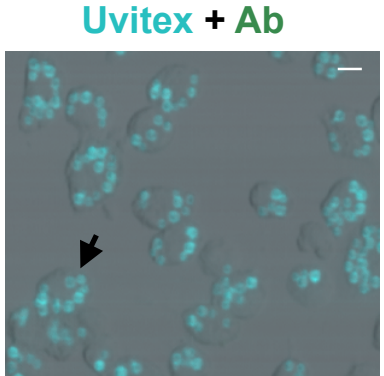
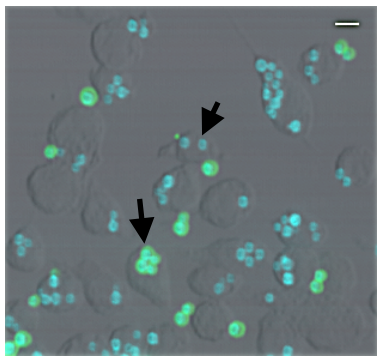
Supplemental Figure 2

A



B





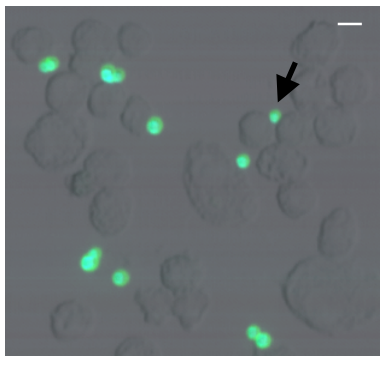


Table 3. Comparison between using Uvitex staining alone or combined with Immunostaining (Ab)

		No yeasts		1-2 Yeasts		>3 Yeasts	
	Well #	Uvitex	Uvitex+Ab	Uvitex	Uvitex+Ab	Uvitex	Uvitex+Ab
Phagocytosis 2 h							
	1	16	15	23	22	62	63
	2	26	25	40	40	34	35
	3	20	20	37	38	43	42
	4	16	17	38	39	45	44
	5	17	17	37	39	45	44
	Mean ± SD	19 ± 4	19 ± 4	35 ± 7	36 ± 8	46 ± 10	46 ± 10
Phagocytosis 15 min							
	1	19	34	36	39	45	27
	2	12	36	30	36	58	27
	3	5	24	18	39	77	37
	4	17	37	37	42	46	21
	5	34	49	42	38	24	13
	Mean ± SD	17 ± 11	36 ± 9*	32 ±9	39 ± 28*	50 ±19	25 ± 9*
Incubation at 4ºC No phagocytosis							
	1	73	99	22	1	5	0
	2	91	99	8	1	1	0
	3	95	100	5	0	0	0
	4	86	100	13	0	2	0
	5	66	94	26	6	7	0
	Mean ± SD	82 ± 12	98 ± 2*	15 ±9	2 ± 2*	3 ±3	0 ± 0*

* p<0.05, Two-tailed Student t-test for paired observations, 95% confidence interval.

Supplemental Figure 2. **Comparison between Uvitex staining alone or combined with Immunostaining (Ab).**

Distinction between internalized and adhered Cn can be made by combining Uvitex with Immunostaining. Extracellular Cn will stain with Uvitex and Alexa 488 conjugated antibody to recognize capsule bound opsonin. A) Illustrative images of staining strategy: a) Uvitex staining of total Cn; b) Extracellular Cn using capsular immunostaining; c) Brightfield image of the same field and d) Merge of the three previous images. Scale bars represent 20 µm. B) Illustrative images showing differentially stained Cn to test the reliability of phagocytic quantification using Uvitex alone (Uvitex) or Uvitex combined with Immunostaining (Uvitex+Ab) in three different conditions. Images are a merge of Uvitex (cyan), Immunostaining (green) and Brightfield (grey). Arrow points to bound Cn and arrowheads points to ingested Cn. Scale bars represent 10 µm. Counts are displayed in Table 3.