

Supplemental File Notes:

All parasite species were incubated with aptamers developed against recombinant wall protein (WP-2) or TA4 antigen-like protein as well as whole *Cyclospora* cell (WC; whole oocyst) aptamers designated S3 and S16.

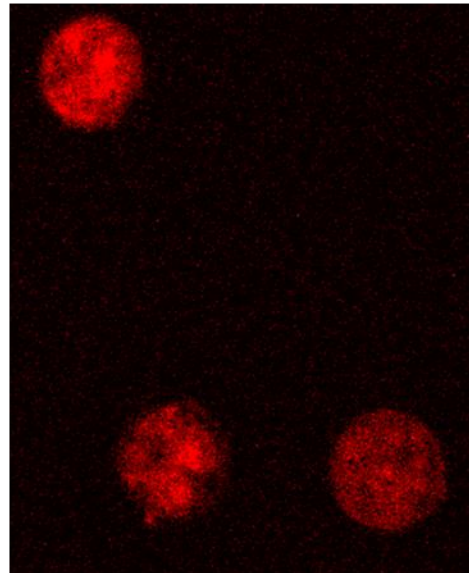
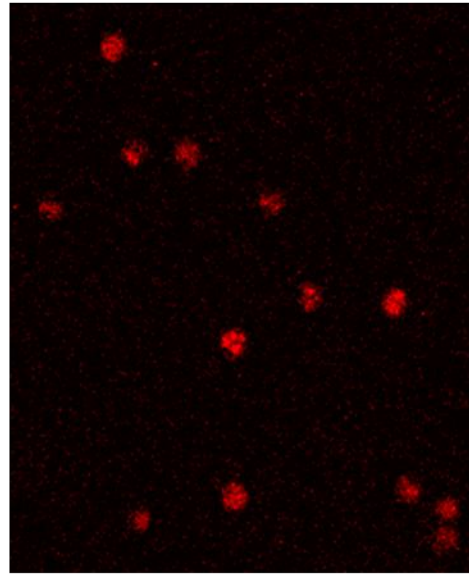
Conclusions:

1. The parasites and oocysts were in a wide range of sizes. To maintain uniformity the magnification was fixed for all samples at 400X total magnification.
2. The density of the oocytes in some of the samples was low. In some fields only one or two oocytes were visible.
3. Some *Cyclospora* aptamers showed very weak positive against non-cognate species.
4. Some *Cyclospora* aptamers showed very weak signals for some aptamers and totally negative for some other aptamers.
5. *Cyclospora cayentanensis* with WP-2-1 aptamer was used uniformly as an assay positive control for each set or family of species samples.
6. Parasites incubated with PBS buffer with no aptamer were used as negative assay control for all samples and never emitted red fluorescence.
7. Optimization of the assays:
 - (a) Each oocyte was incubated with different incubation time (10, 30, 60 min) to rule out the possibility of insufficient incubation time being the reason for negative results (i.e., to eliminate false negatives).
 - (b) The oocytes were incubated with decreasing concentrations of aptamer to rule out the possibilities of non-specific binding of biotin-labelled aptamer (100, 50, and 30 μ l of 1 ml stock aptamers at $\sim$ 1.5 mg/ml) as the cause of positive results (i.e., to eliminate false positives).

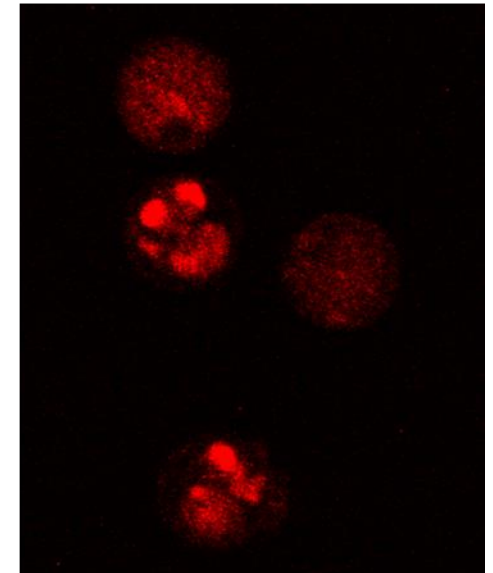
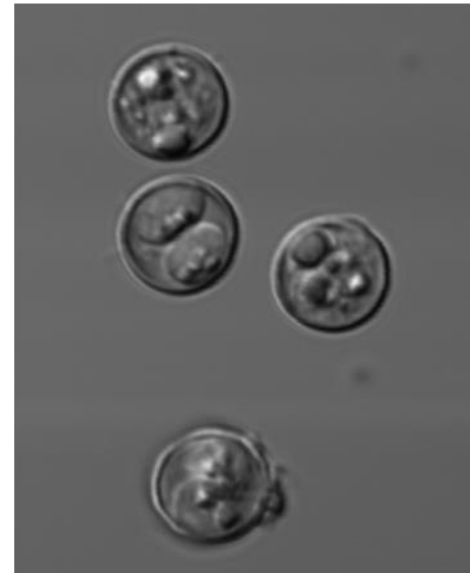
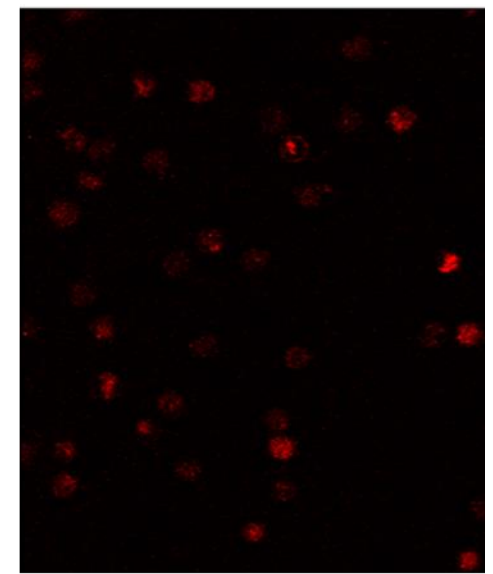
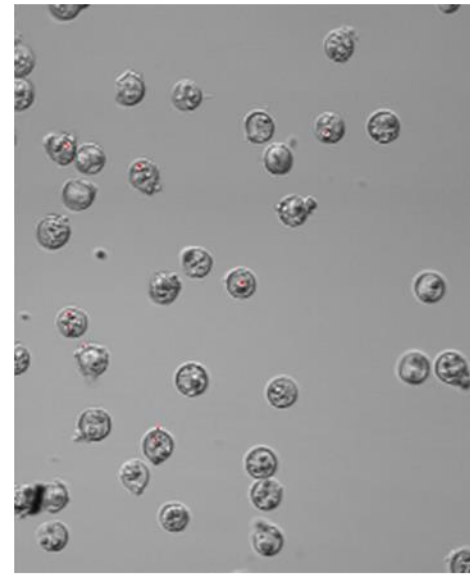
Cyclospora cayetanensis

Aptamers:

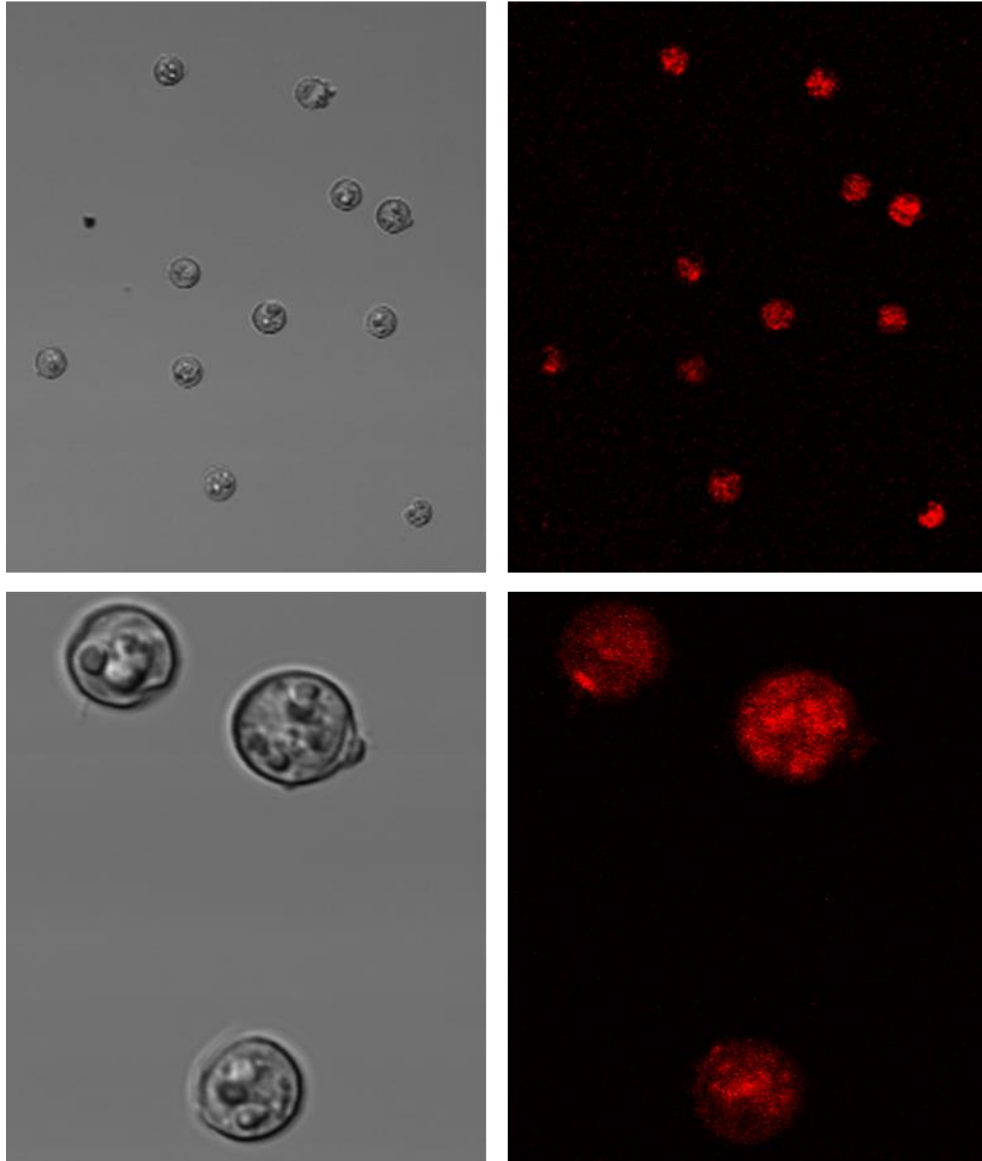
WP2-1



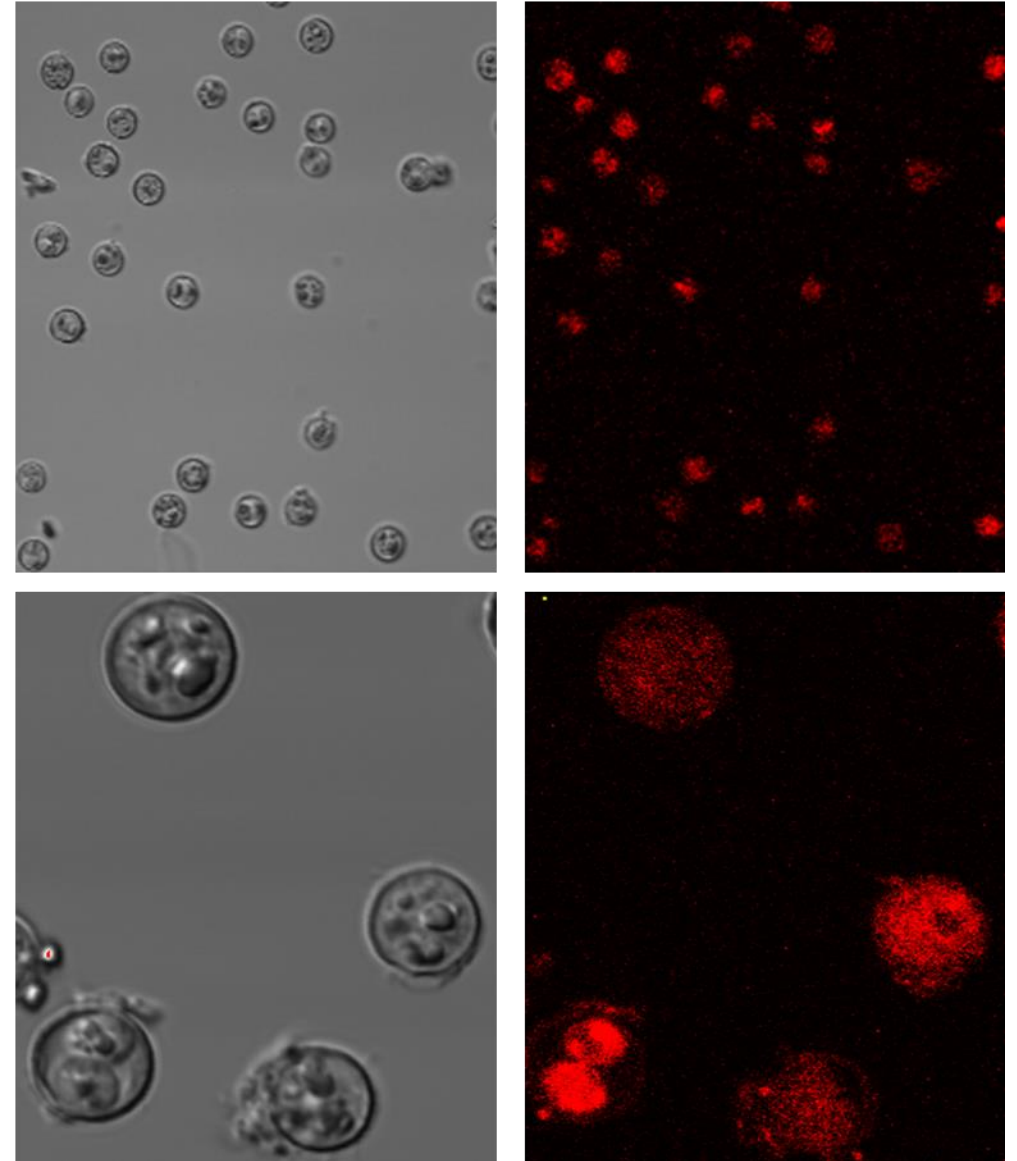
WP2-4



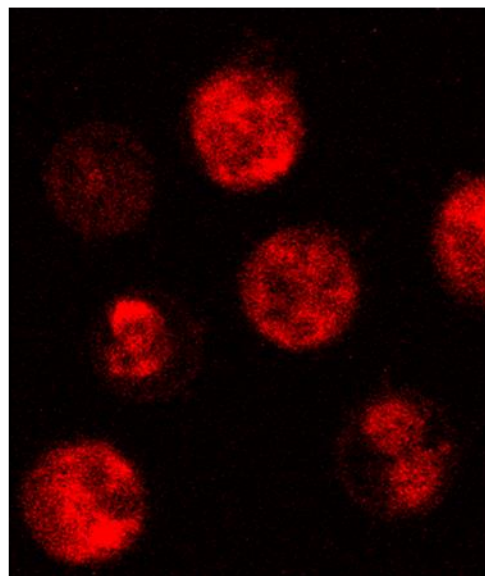
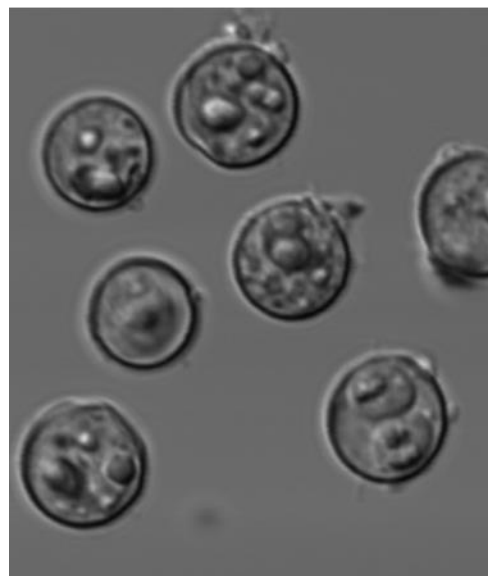
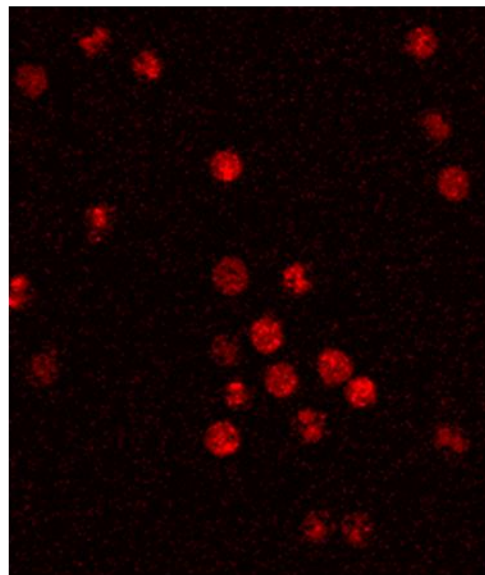
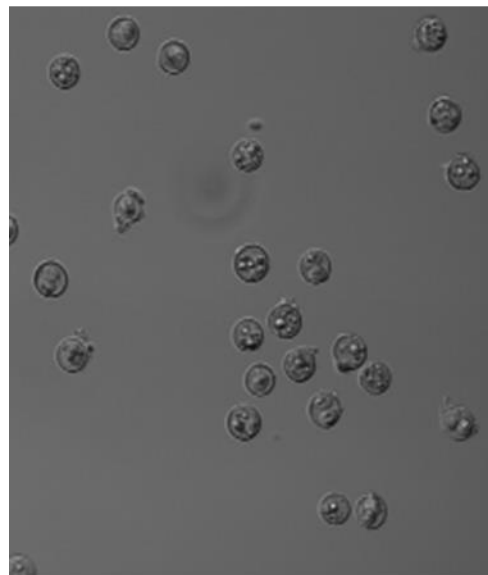
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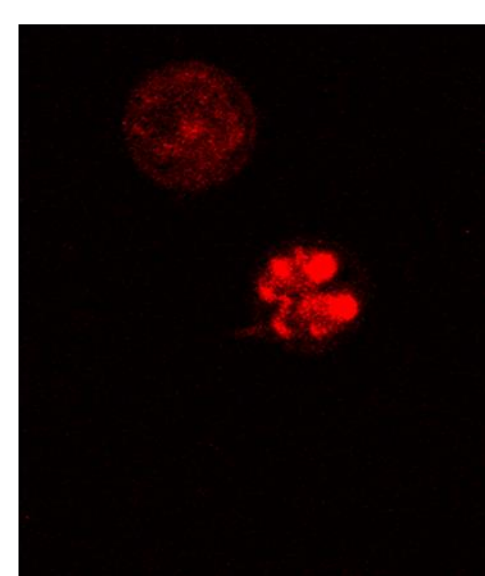
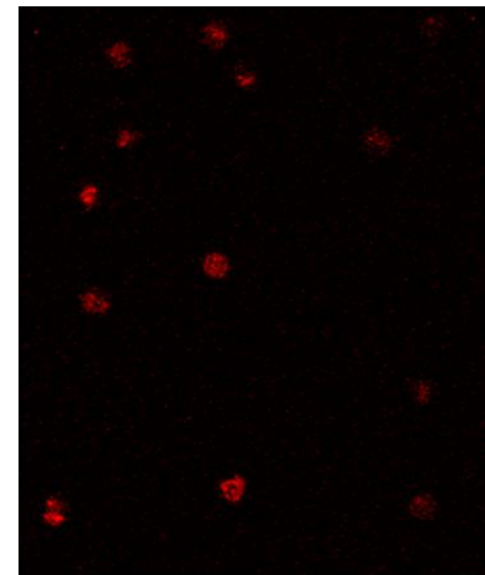
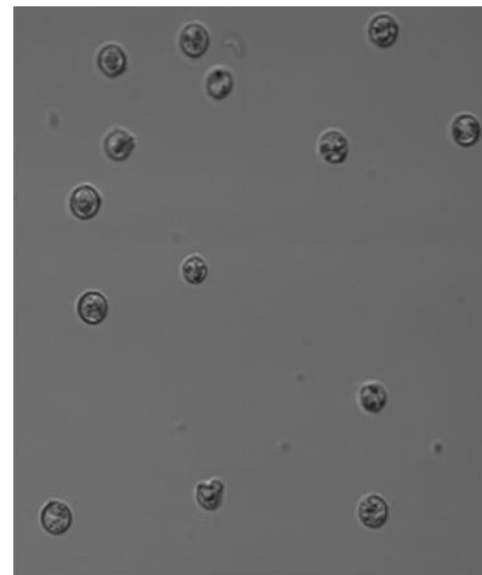
WP2-3



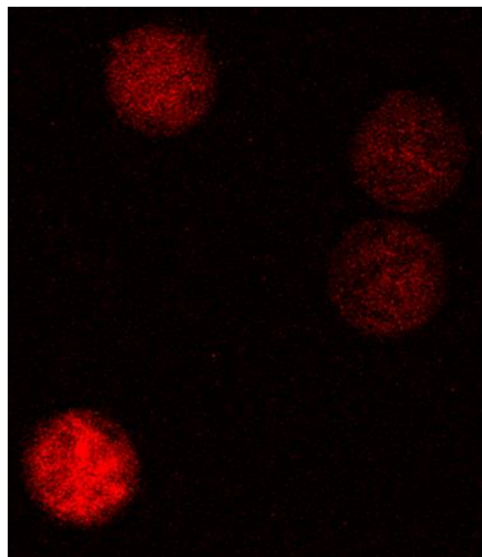
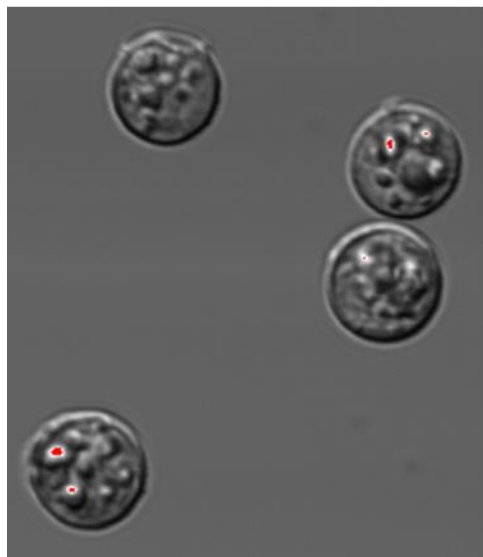
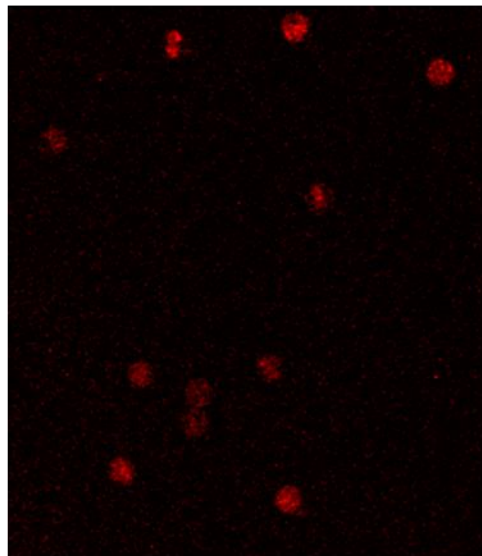
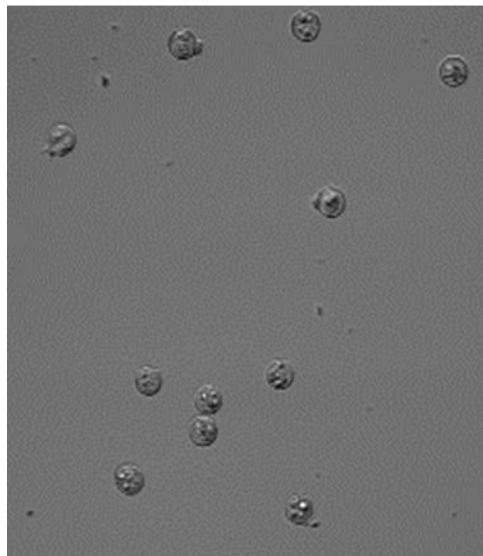
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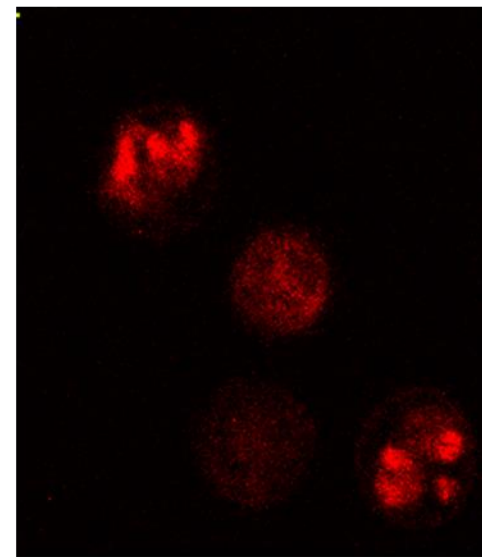
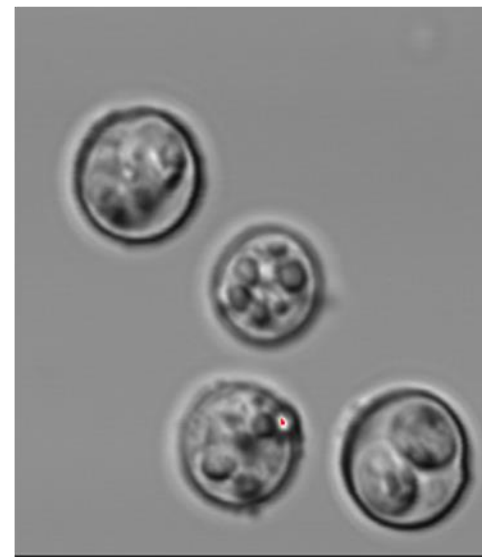
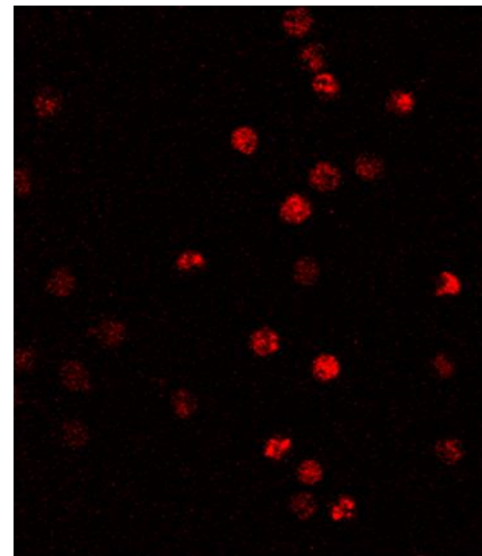
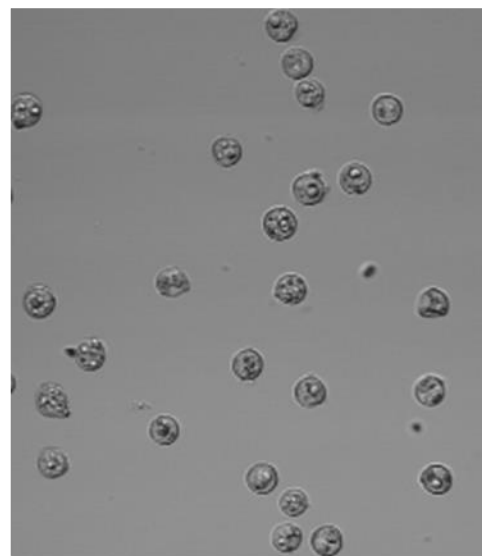
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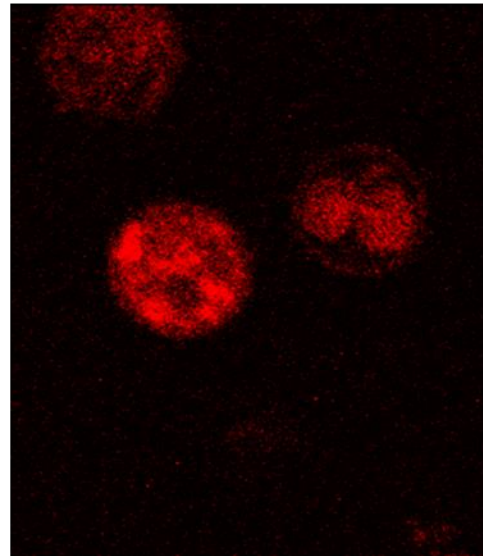
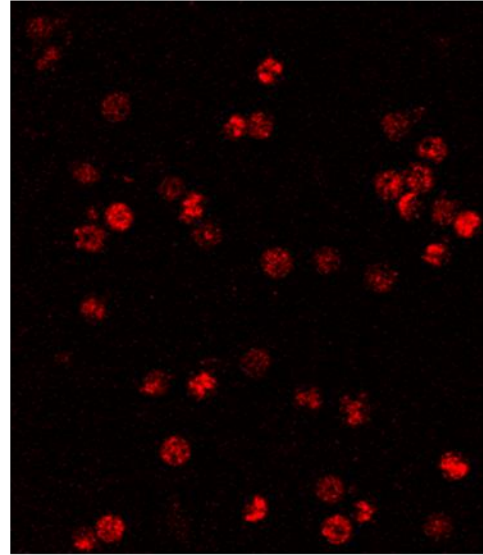
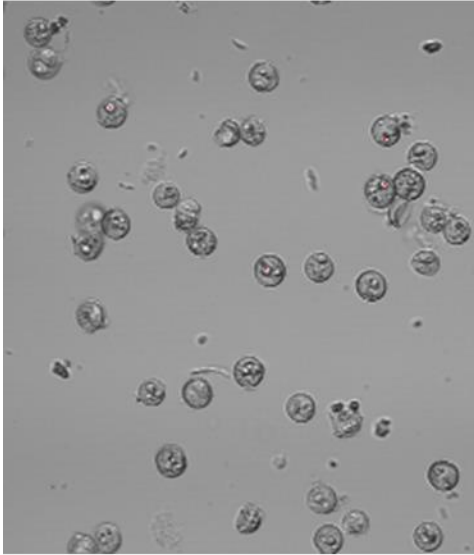
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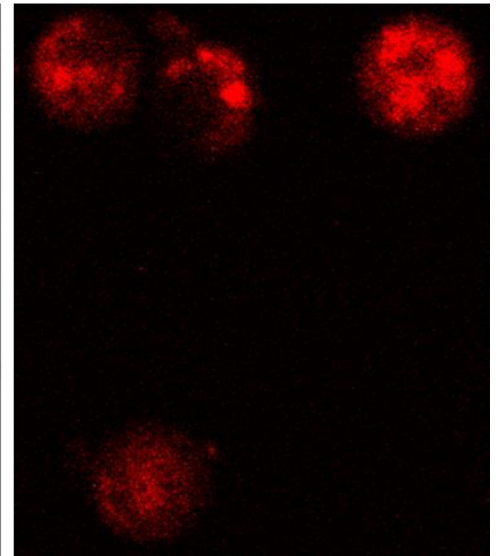
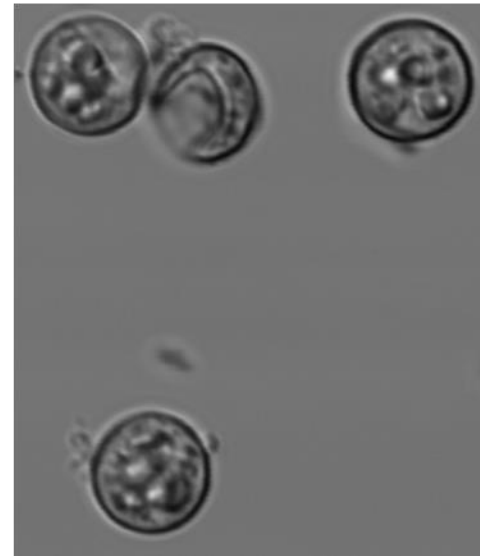
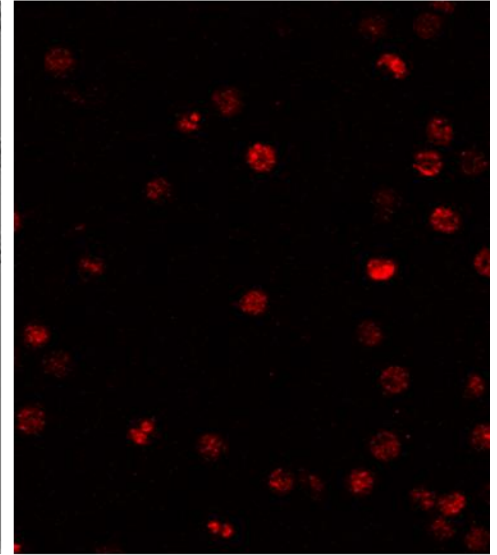
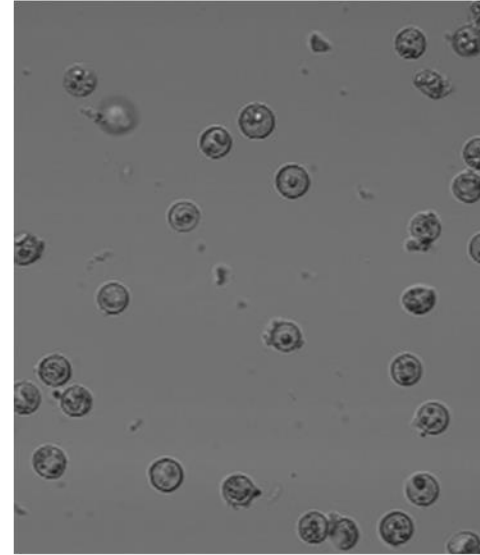
TA4-4



WC-S16



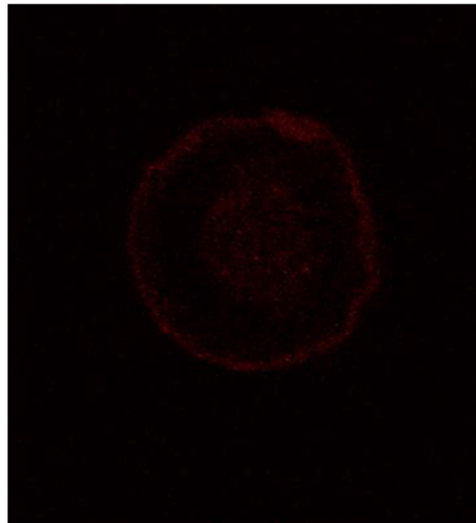
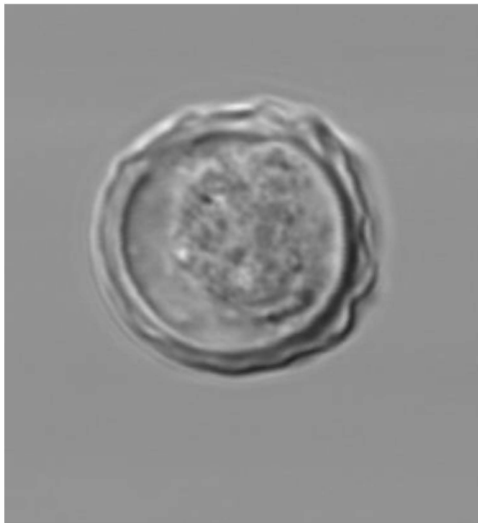
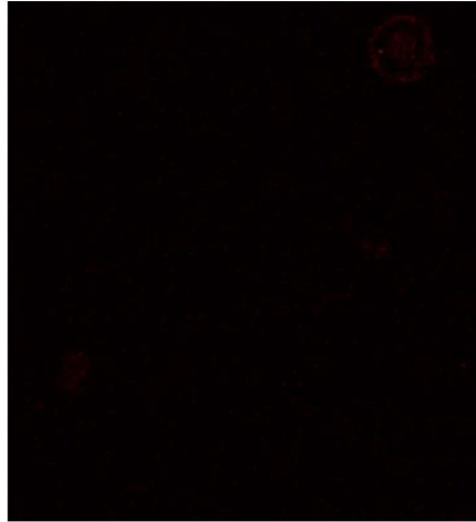
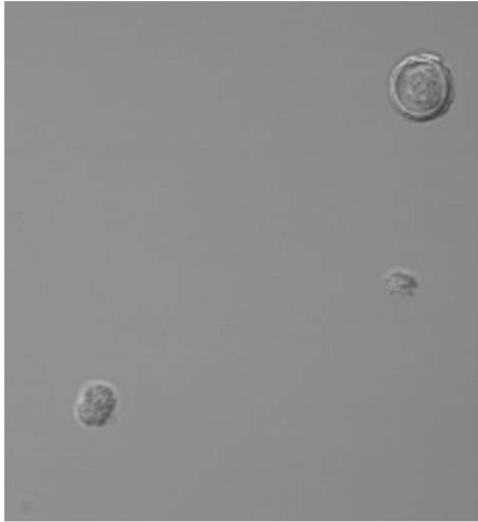
WC-S3



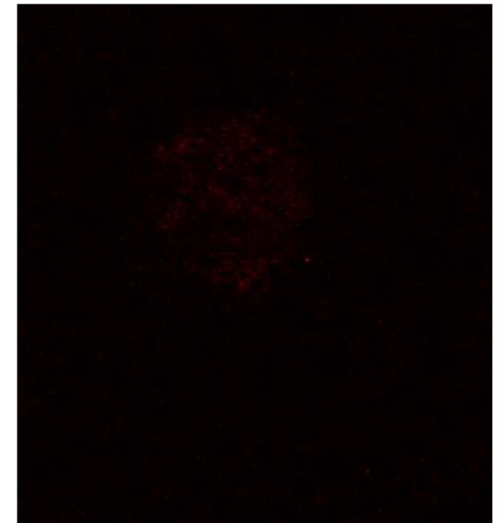
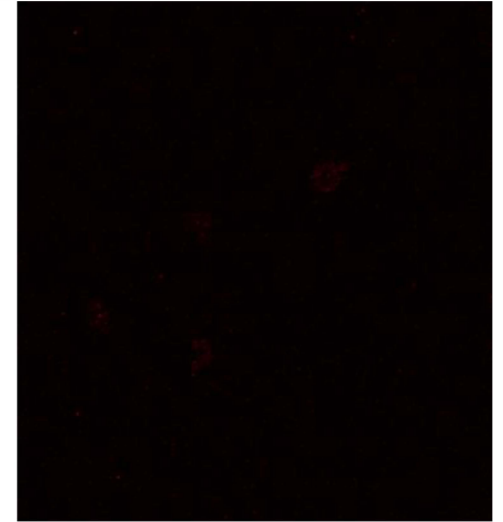
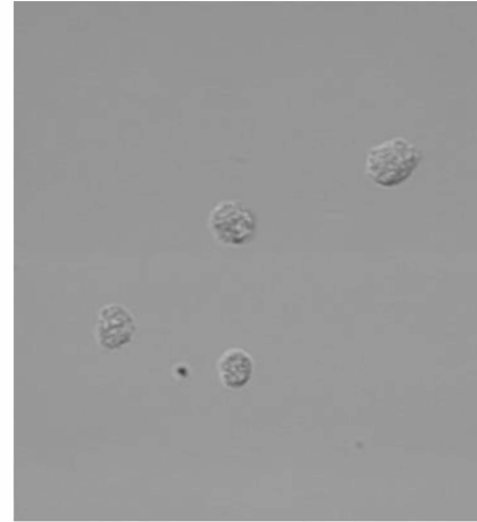
Conclusion: Cyclospora cayatenensis showed strong positive fluorescence signals with all ten different aptamers.

Acanthamoeba Species

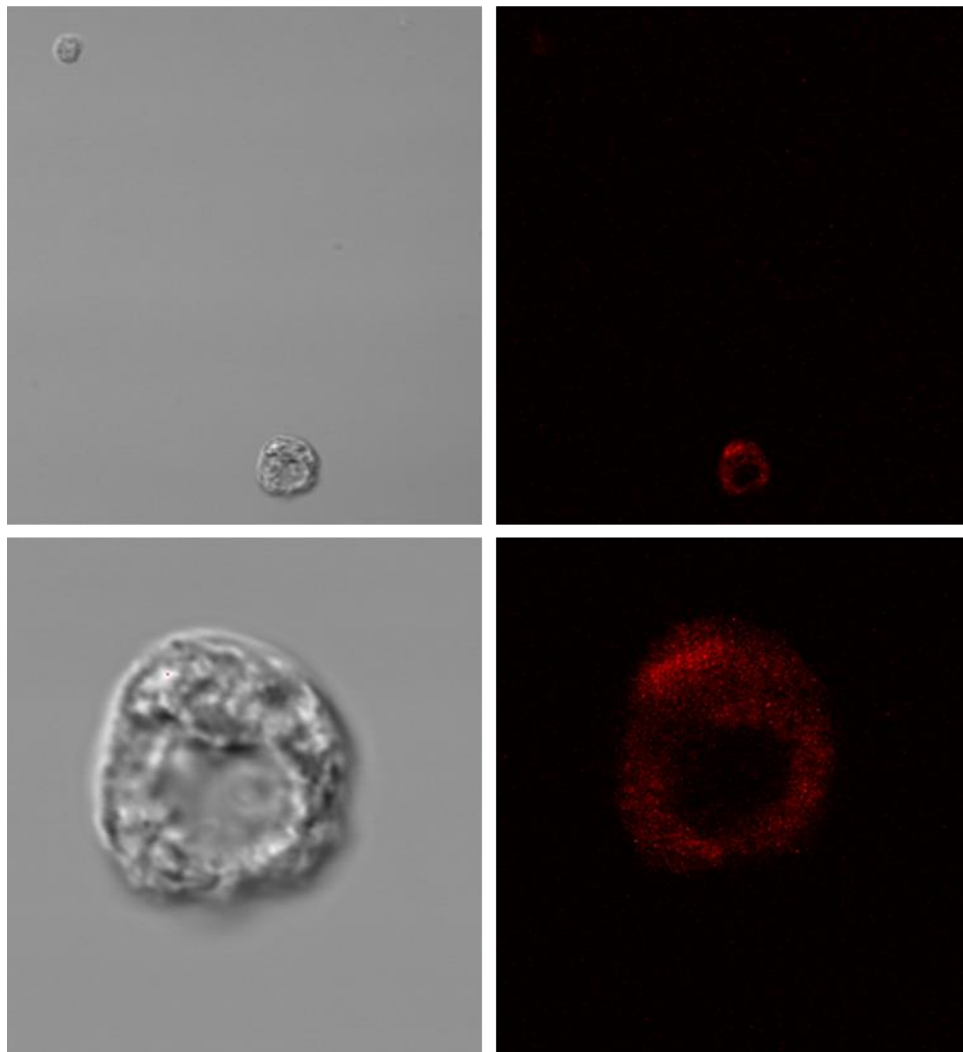
WP2-1



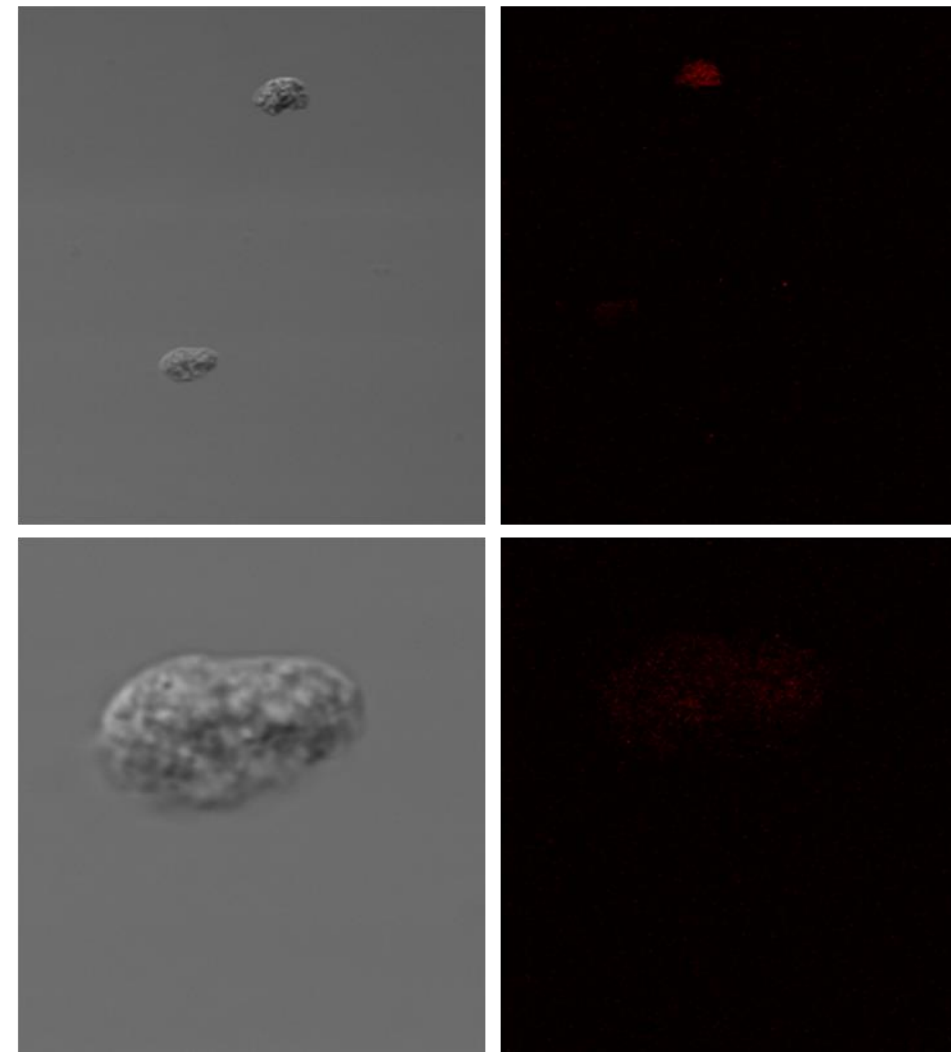
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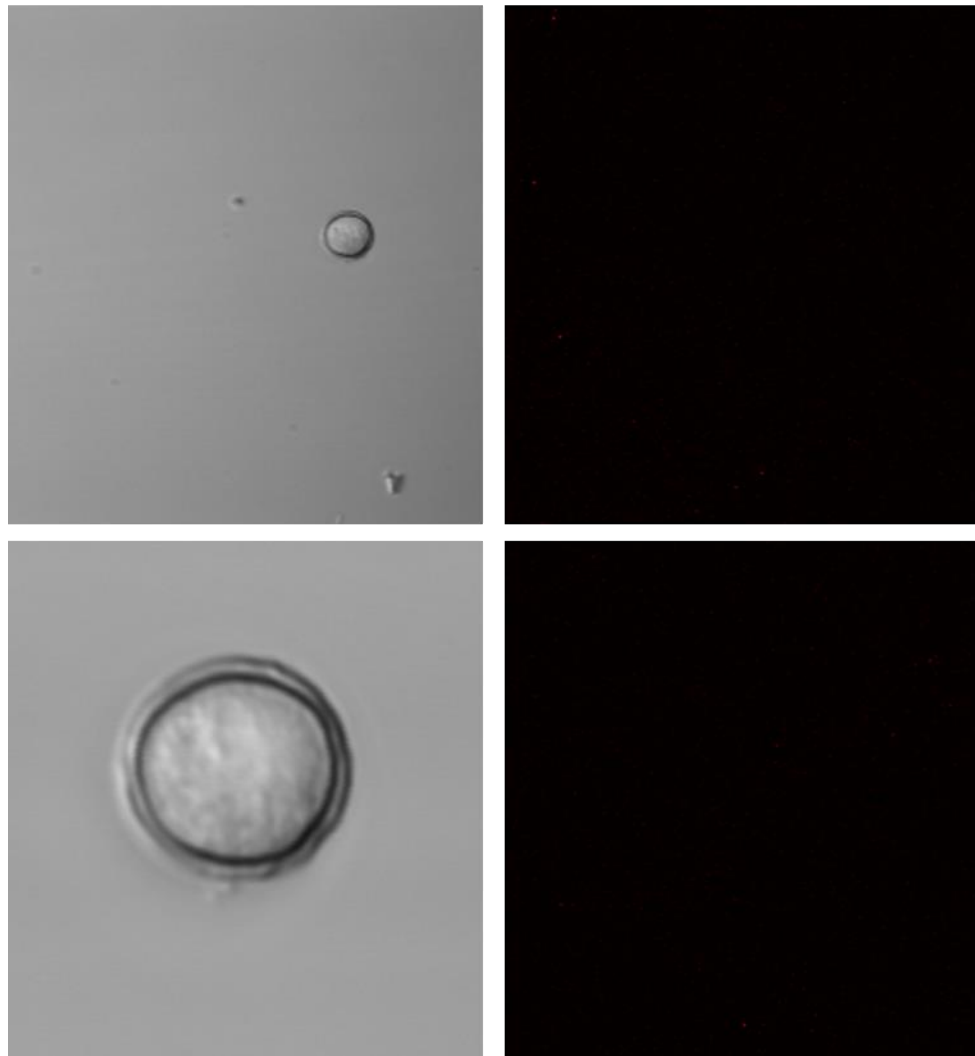
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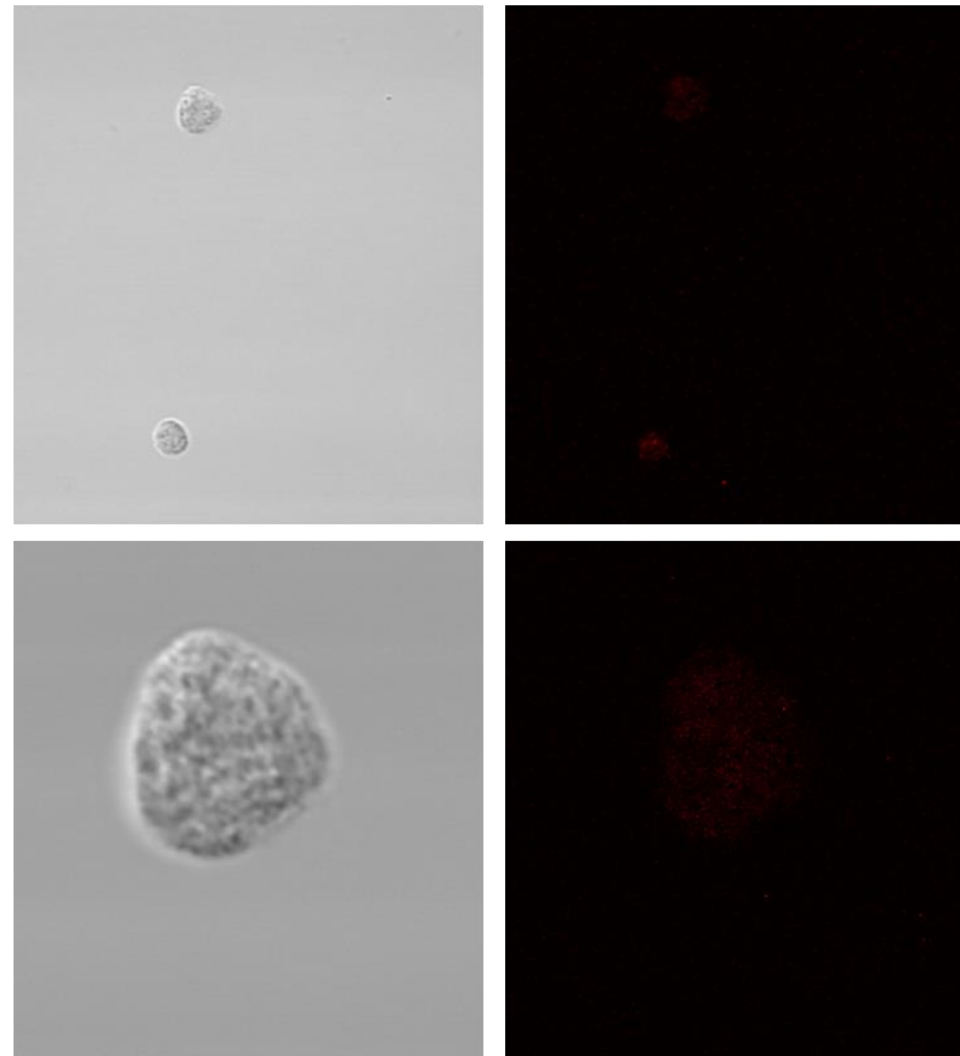
WP2-3



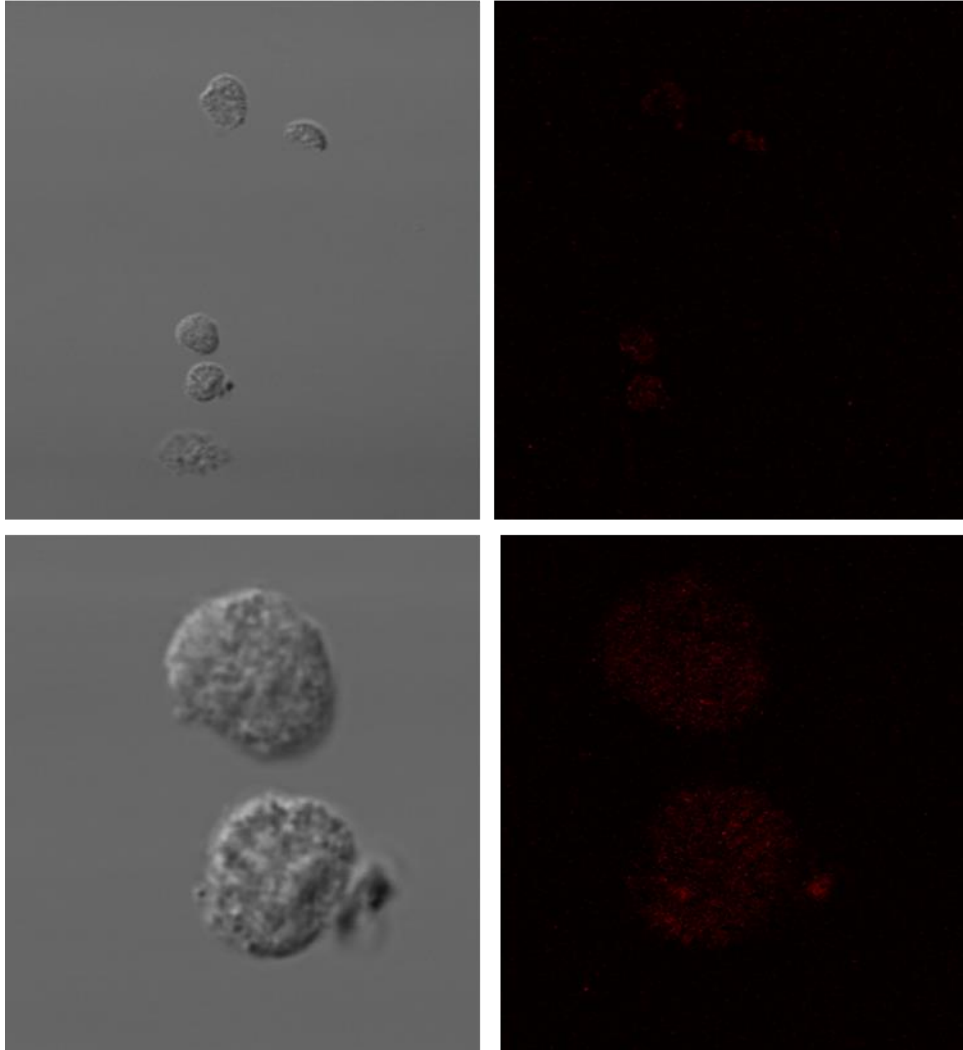
TA4-1



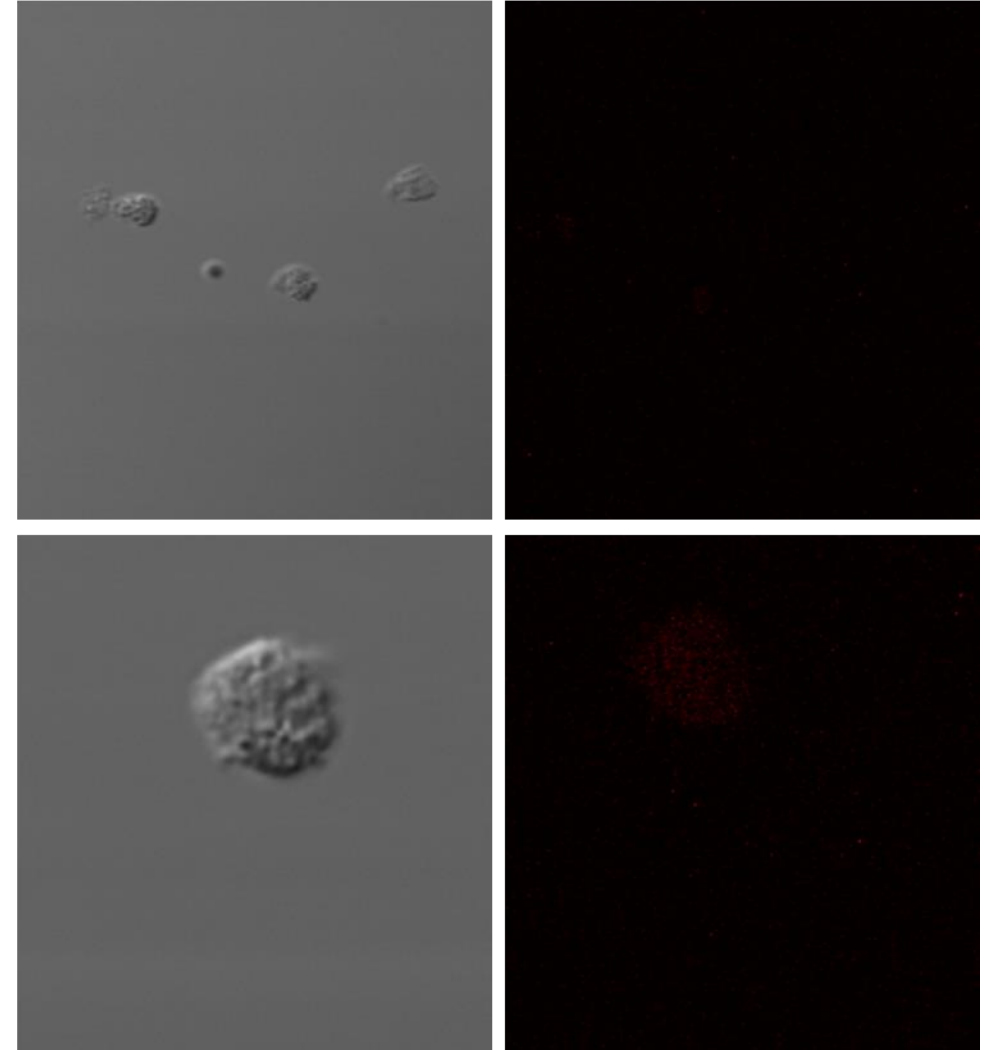
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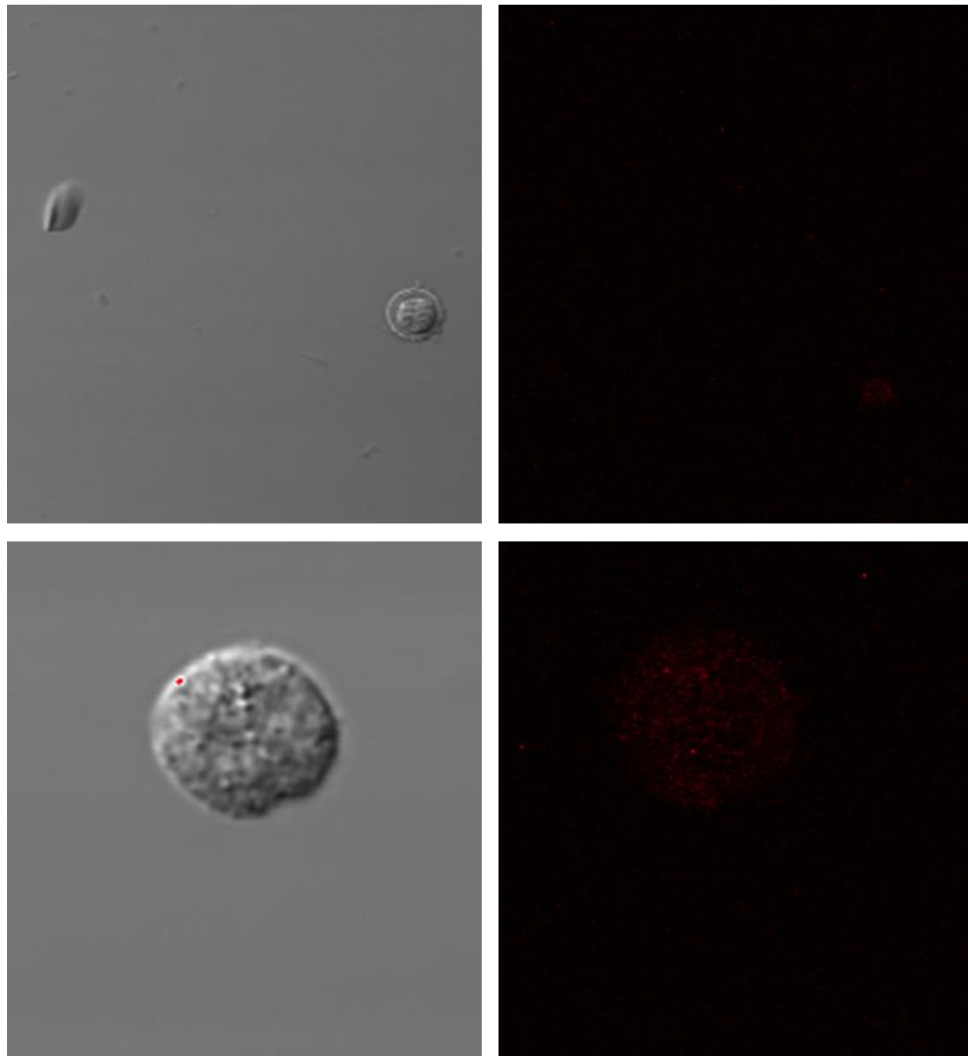
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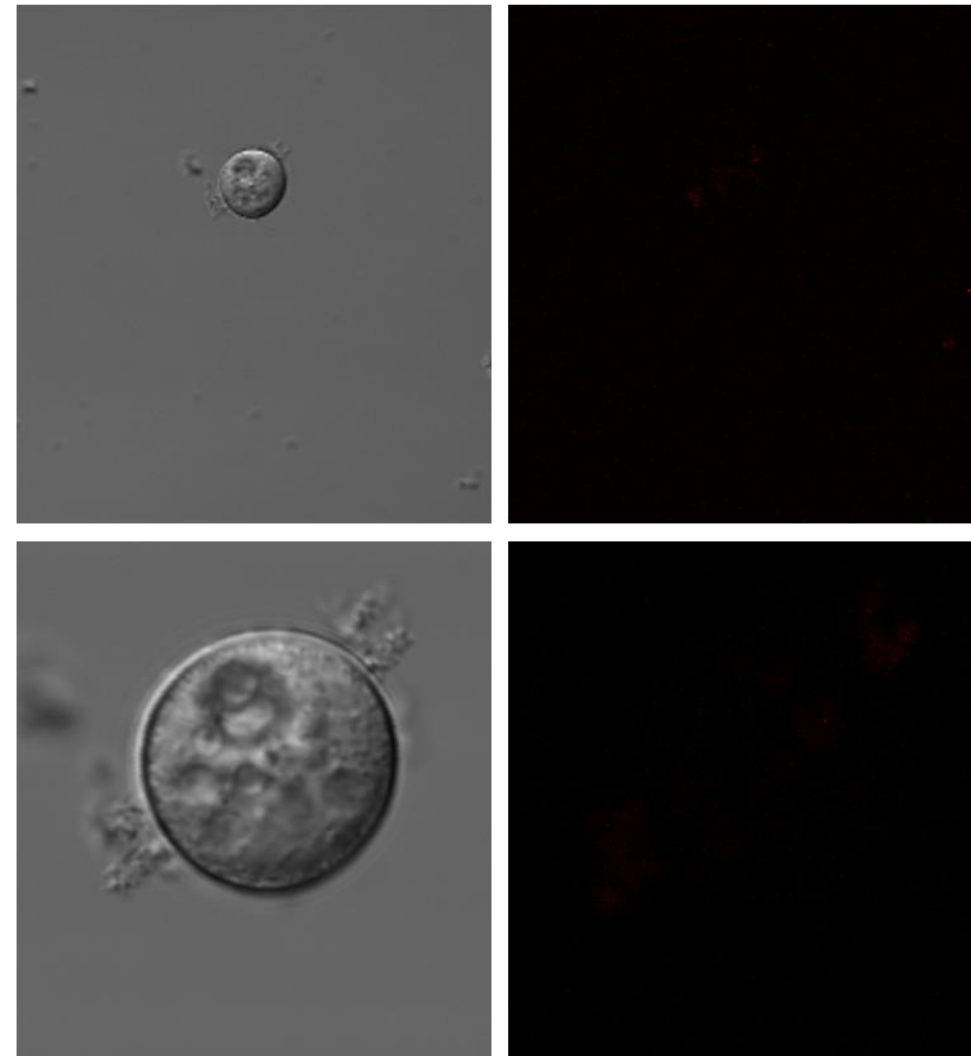
TA4-4



Whole Cyclo S16

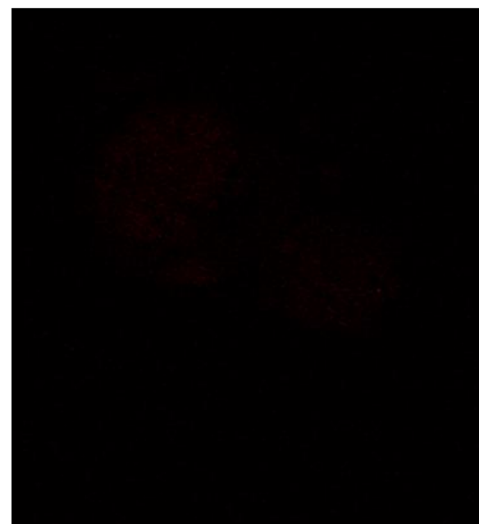
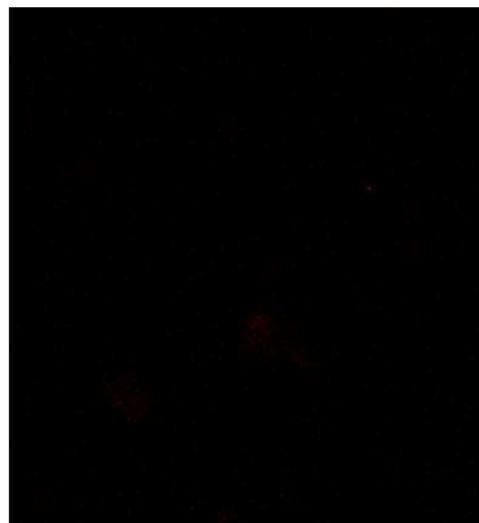
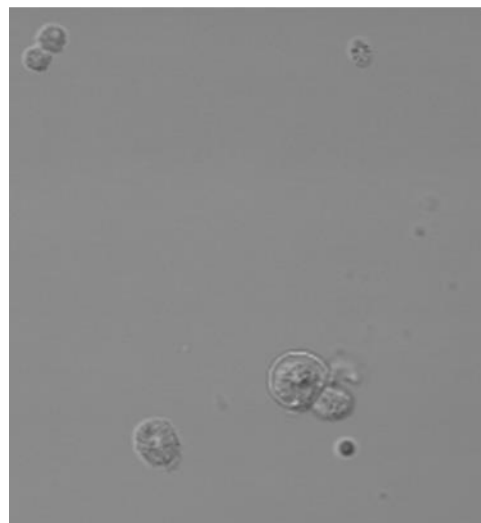


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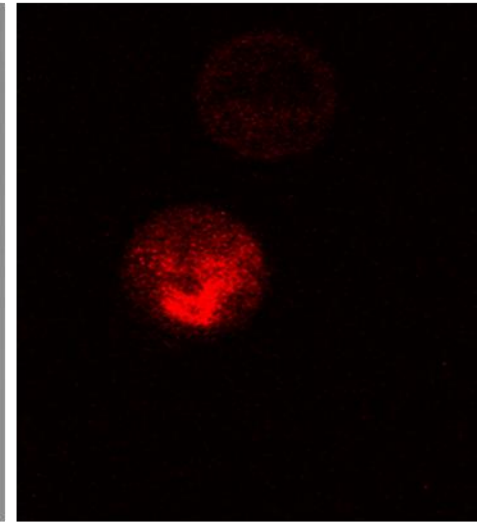
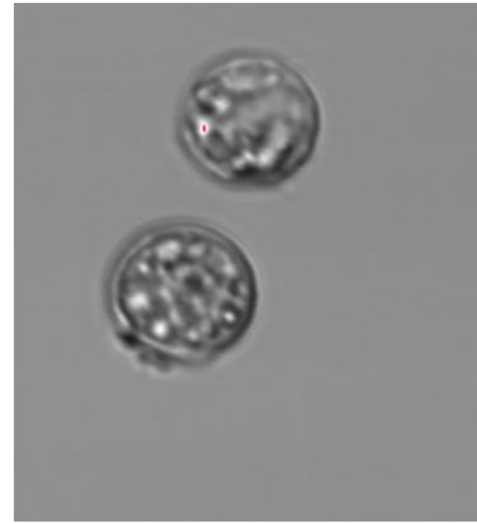
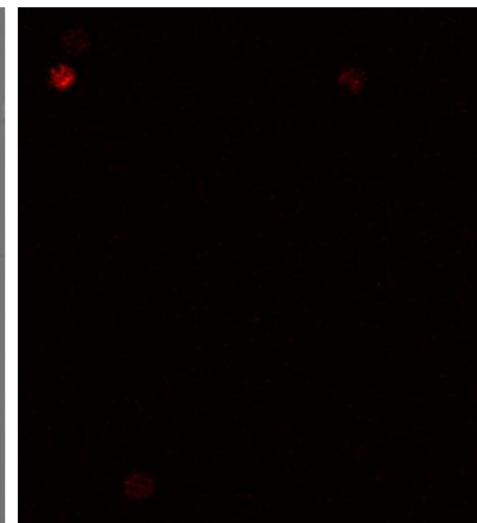
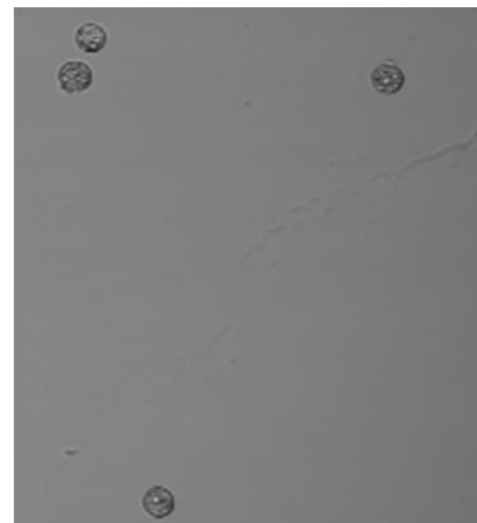


Assay Controls

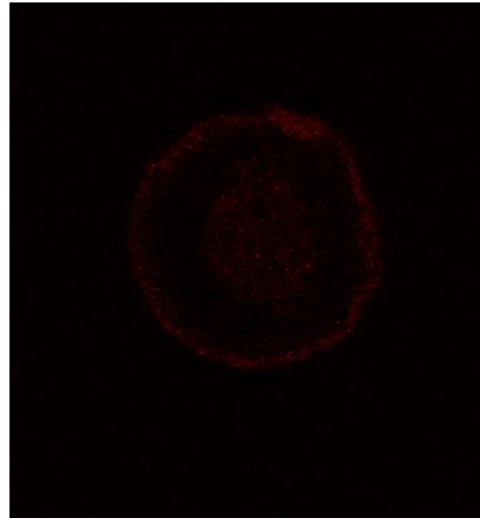
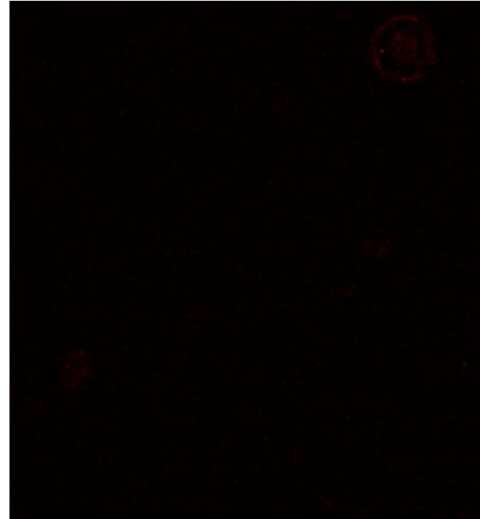
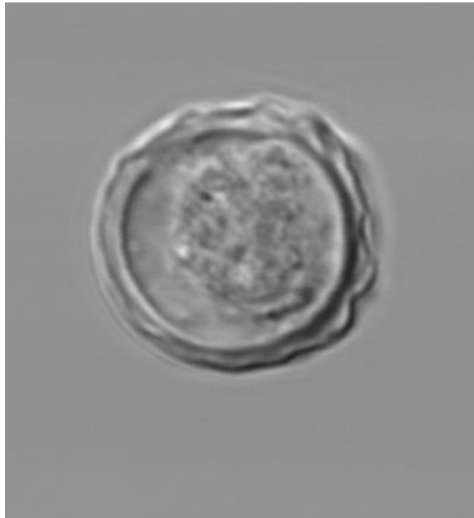
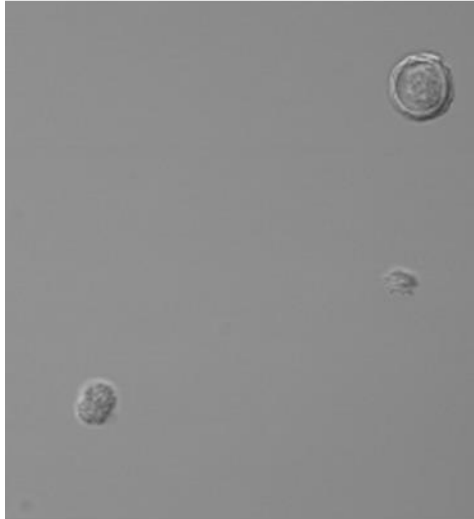
**No Aptamer
(-)ve Control**



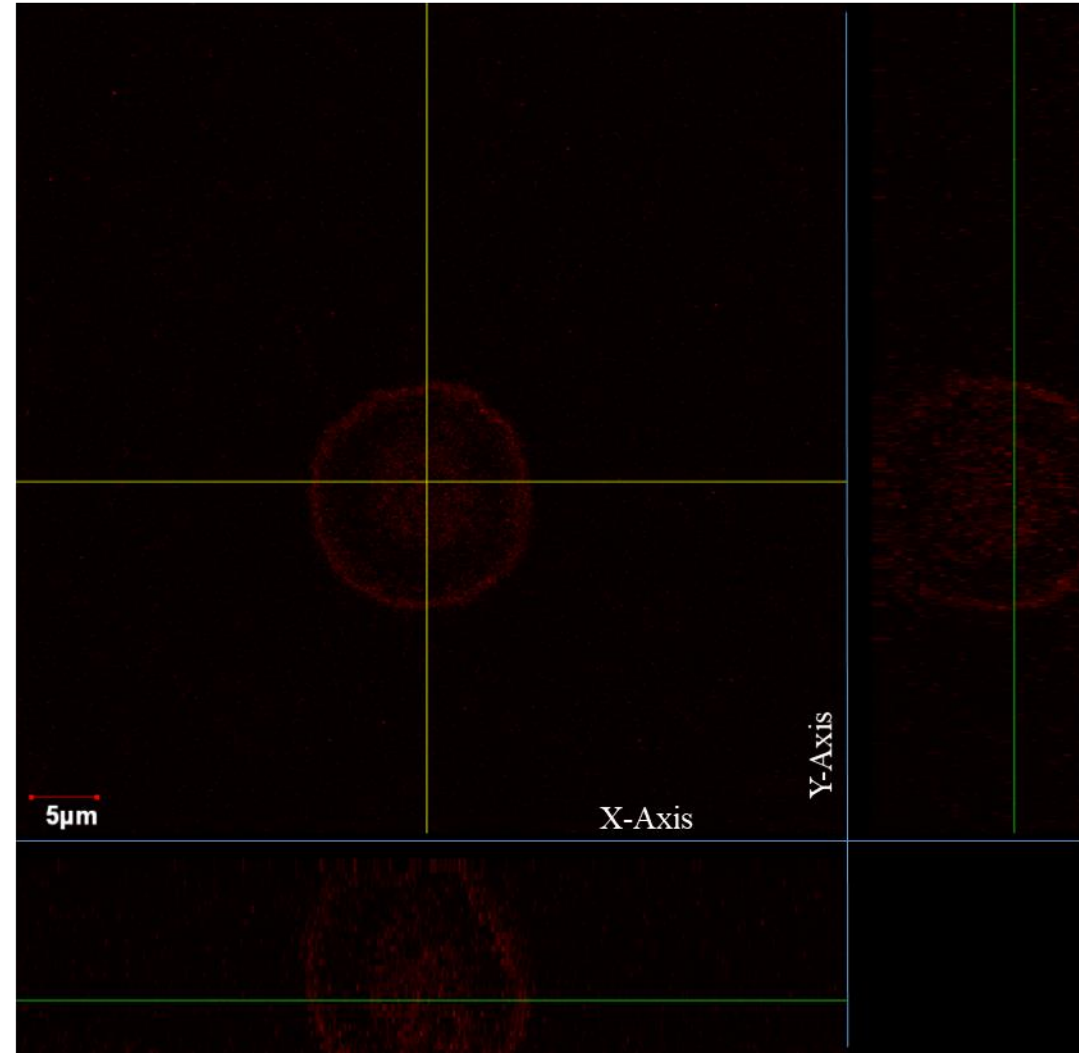
**WP2-1 + Cyclospora
(+)ve Control**



WP2-1



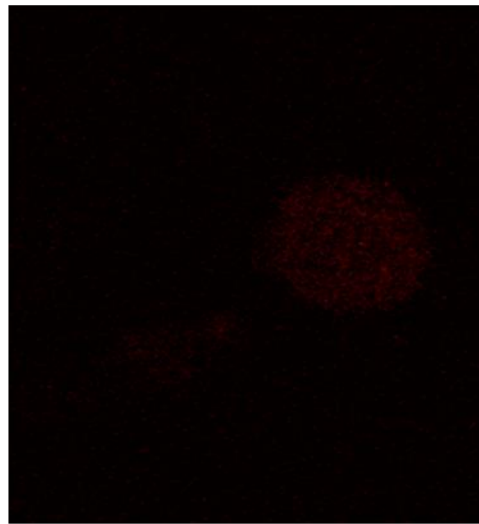
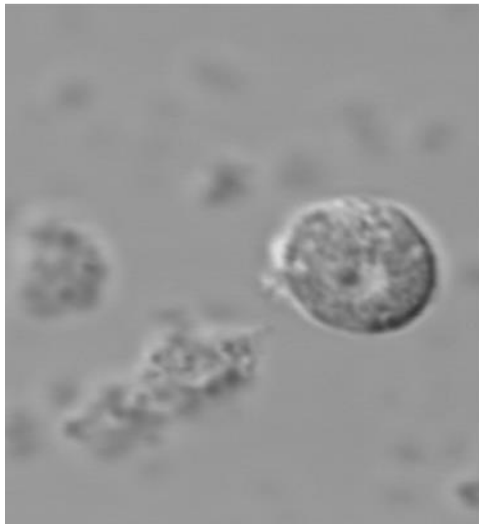
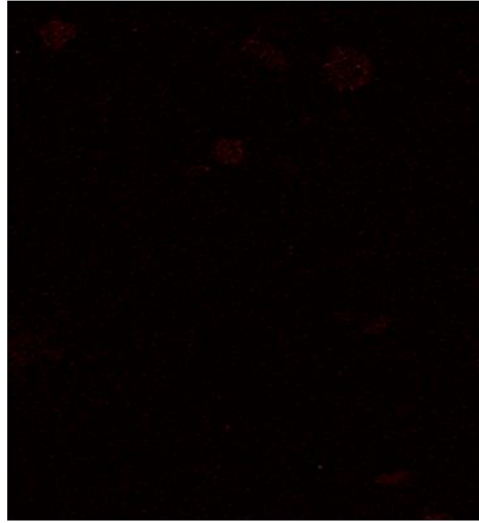
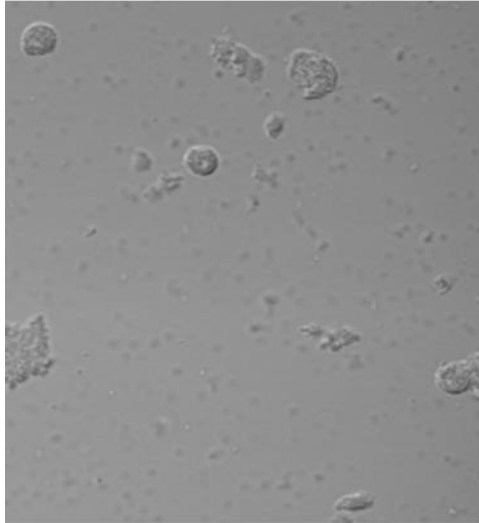
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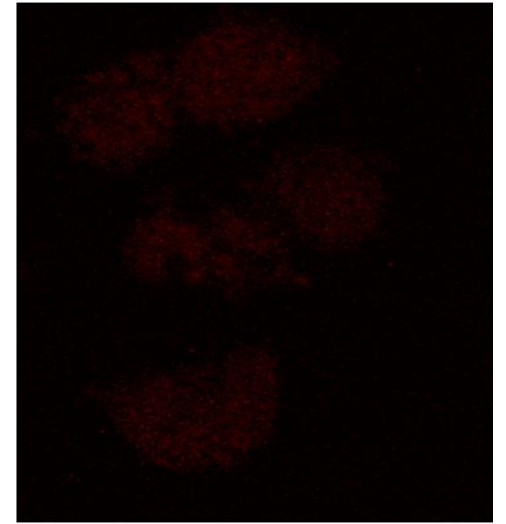
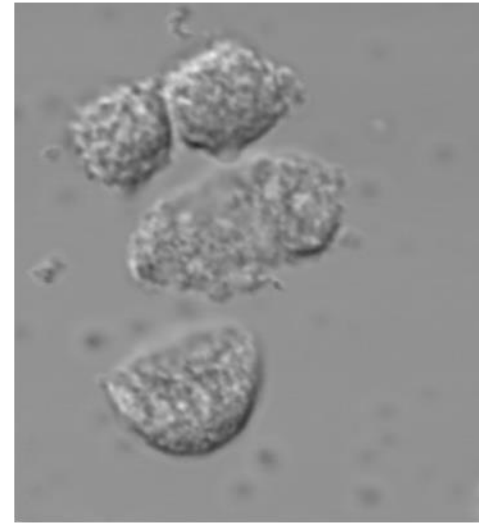
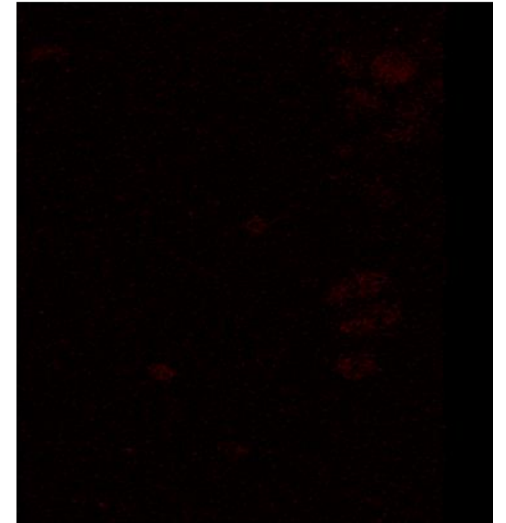
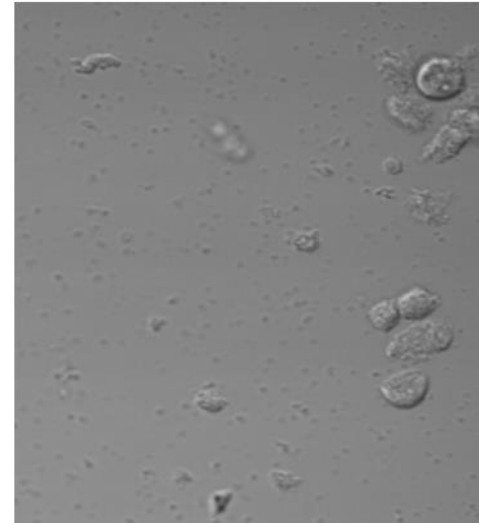
Conclusion: Acanthamoeba species showed weak positive signals with most of the aptamers. WP2-1 and WP2-2 aptamers showed a slight increase in signals over other aptamers.

Balamuthia Mandrillaris

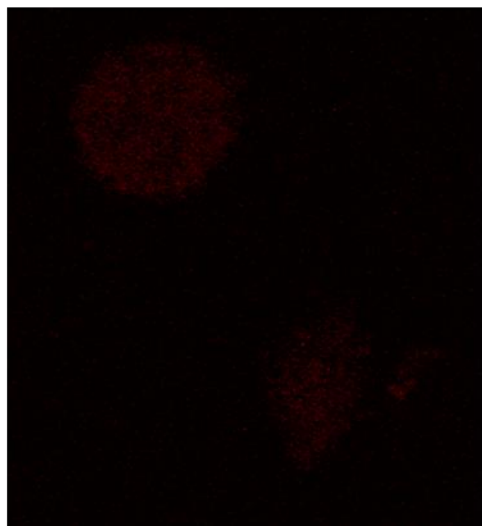
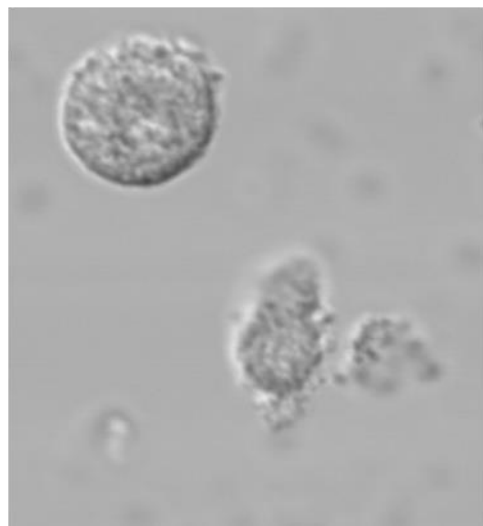
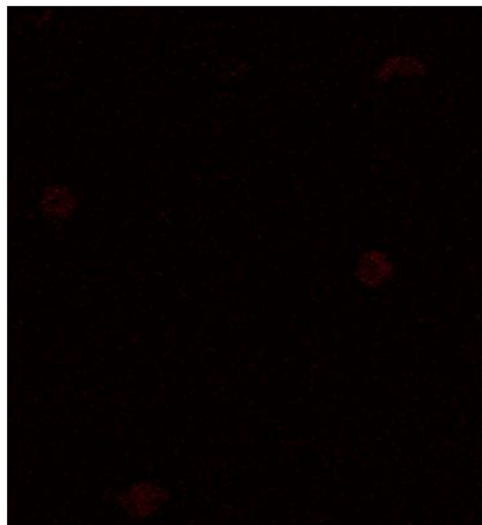
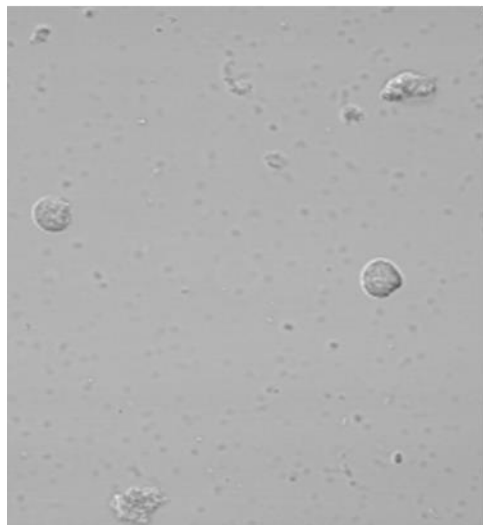
WP2-1



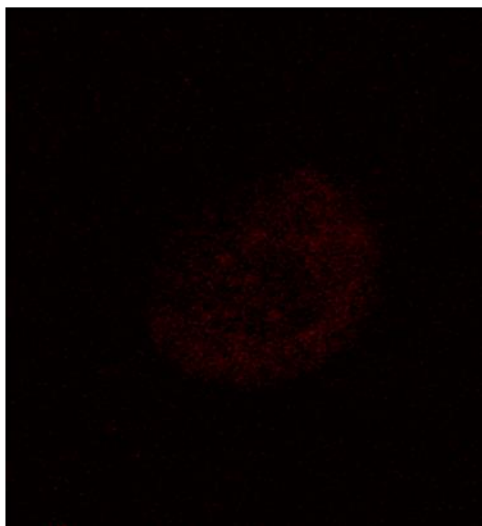
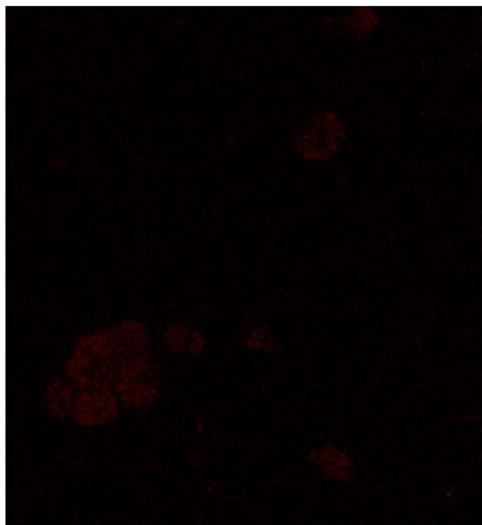
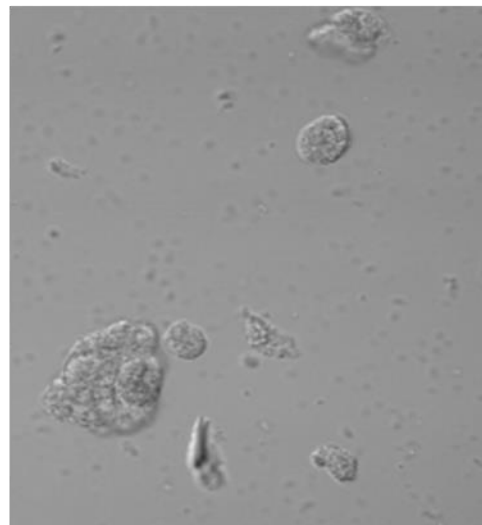
WP2-4



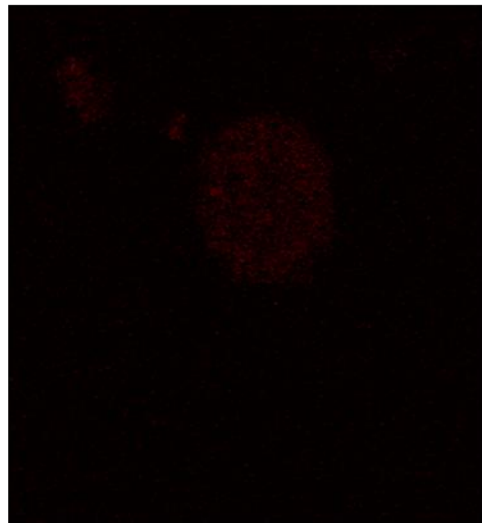
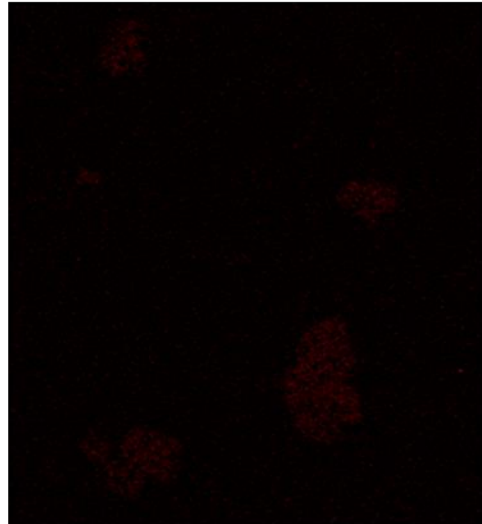
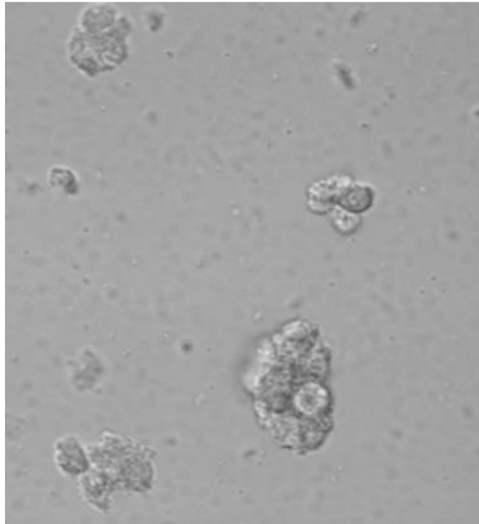
WP2-2



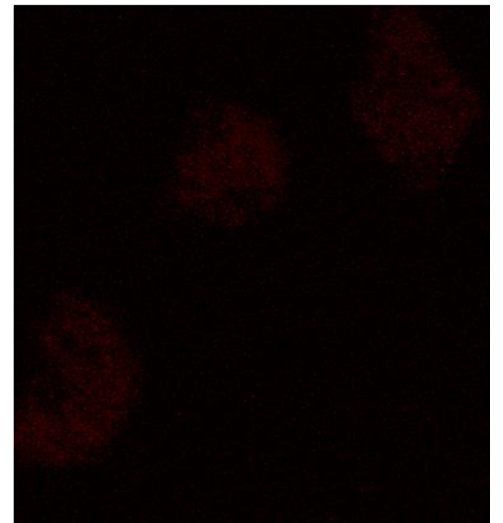
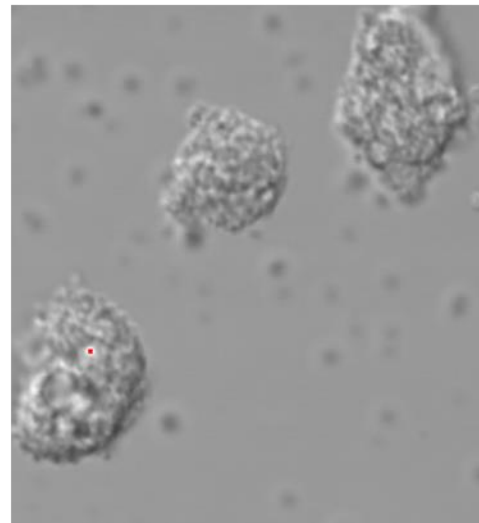
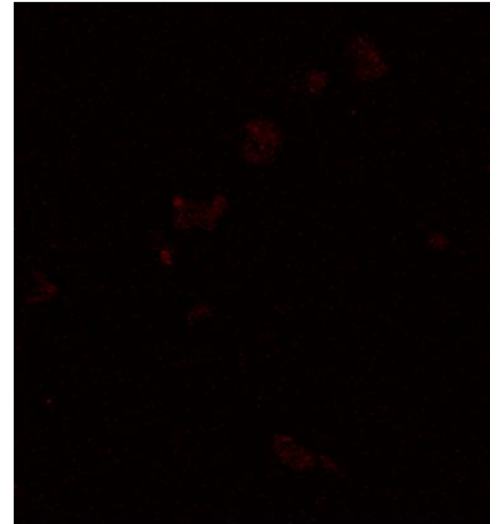
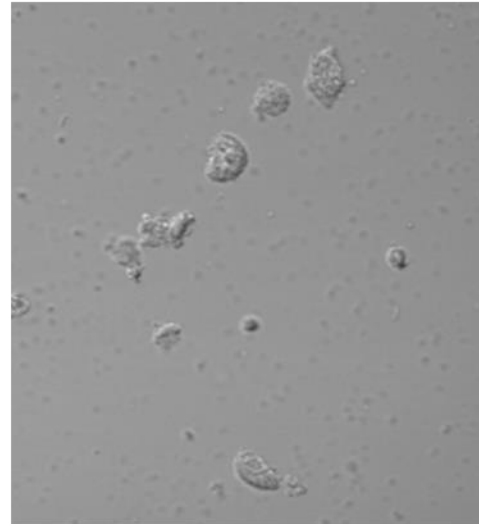
WP2-3



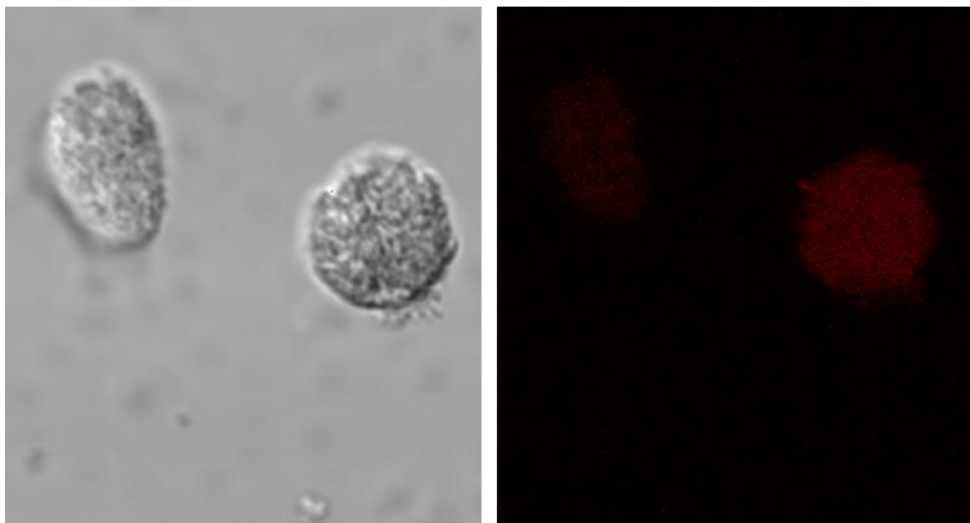
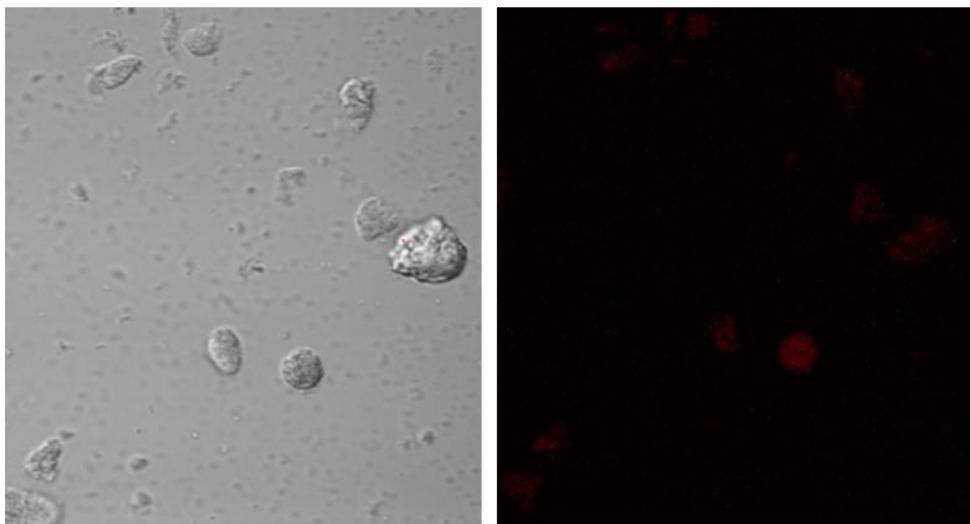
TA4-1



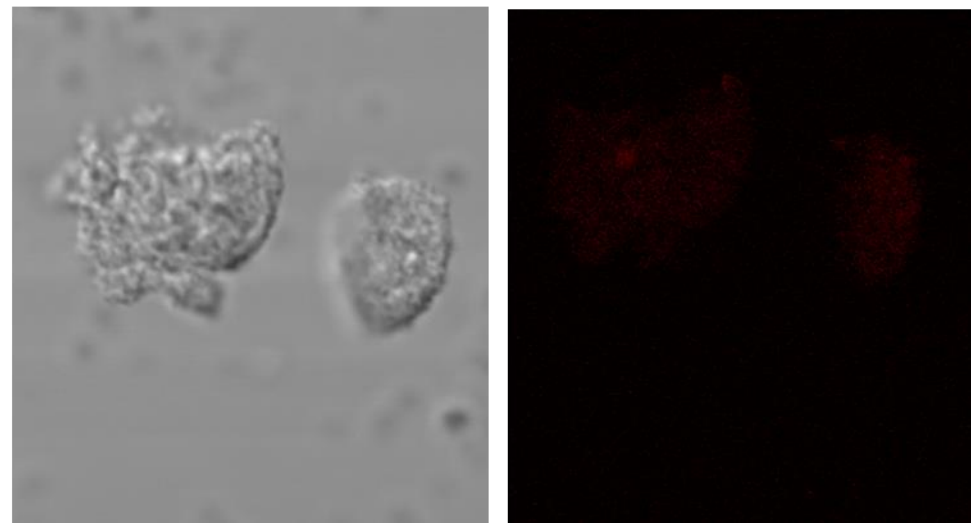
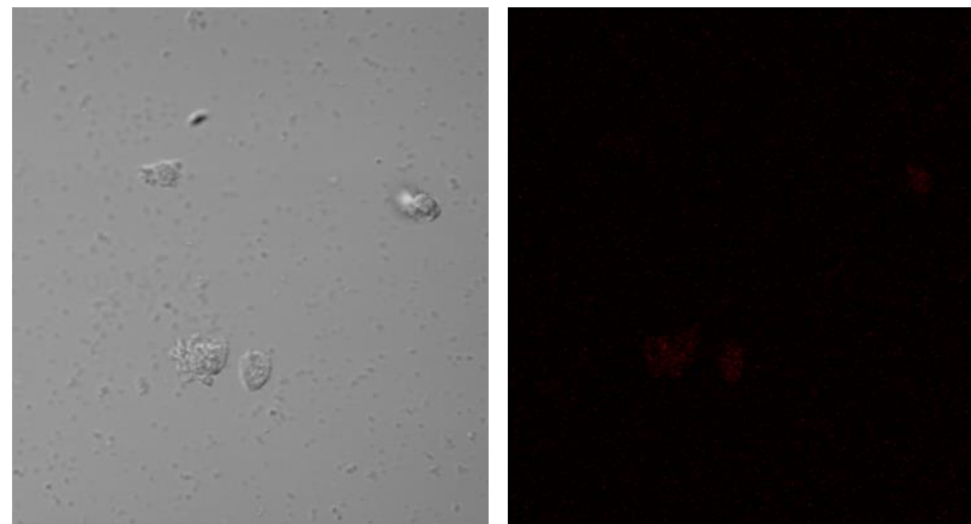
TA4-3



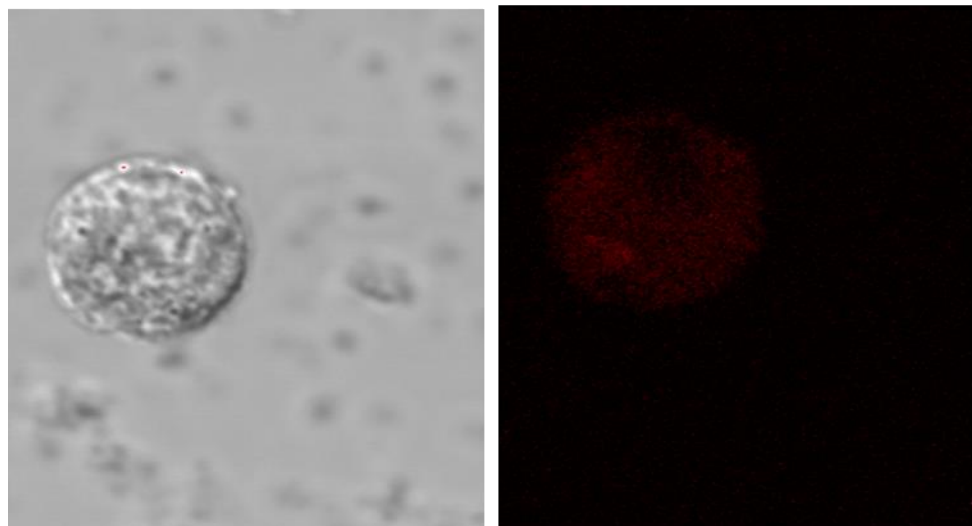
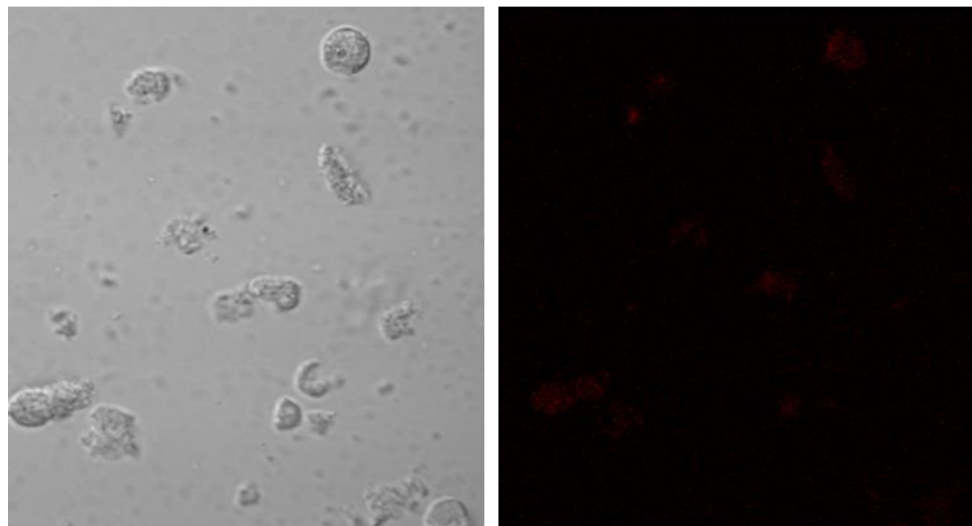
TA4-2



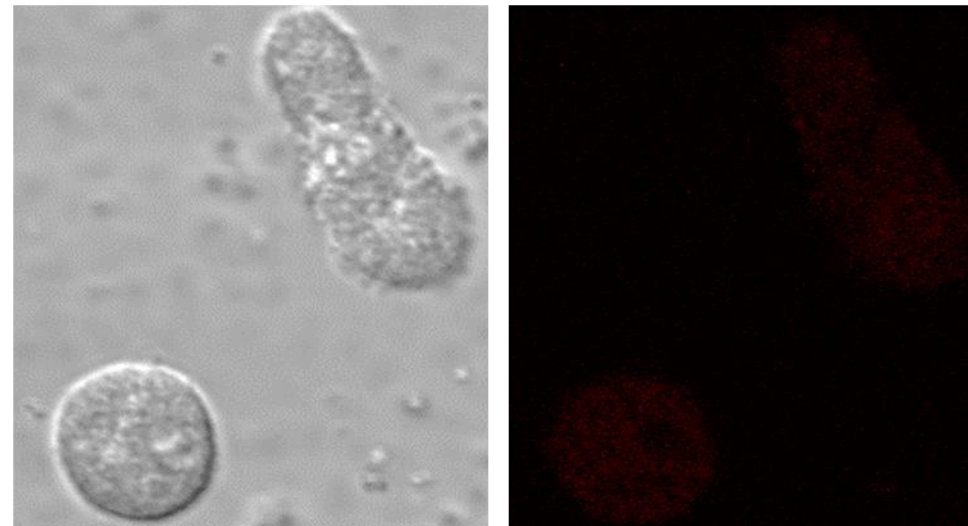
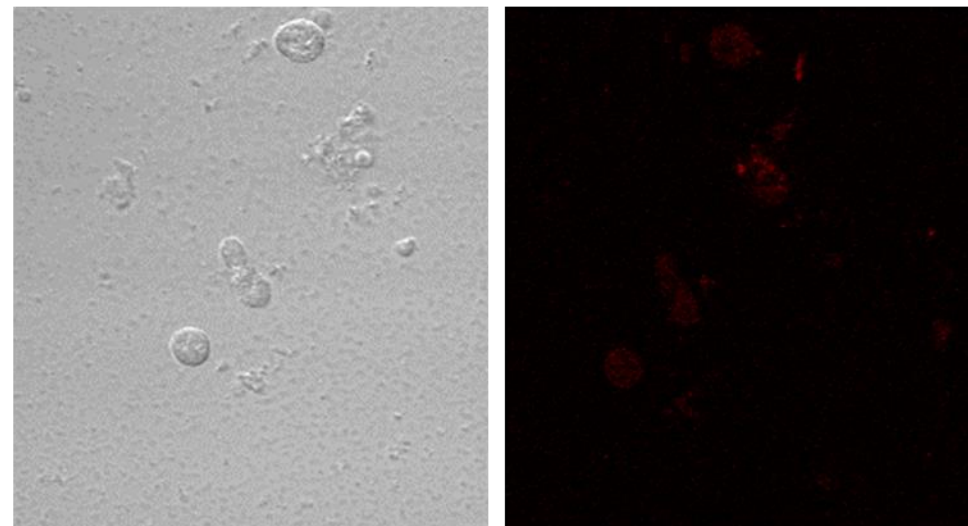
TA4-4



Whole Cyclo S16

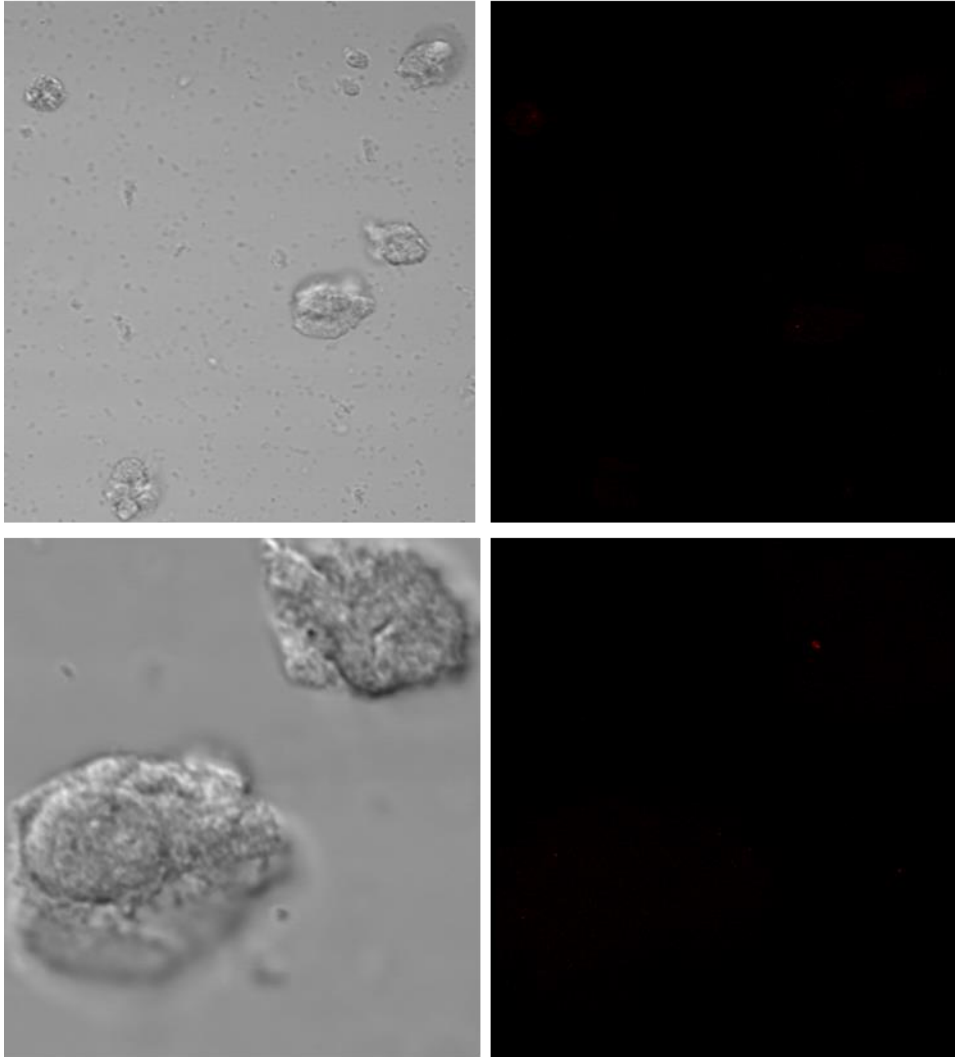


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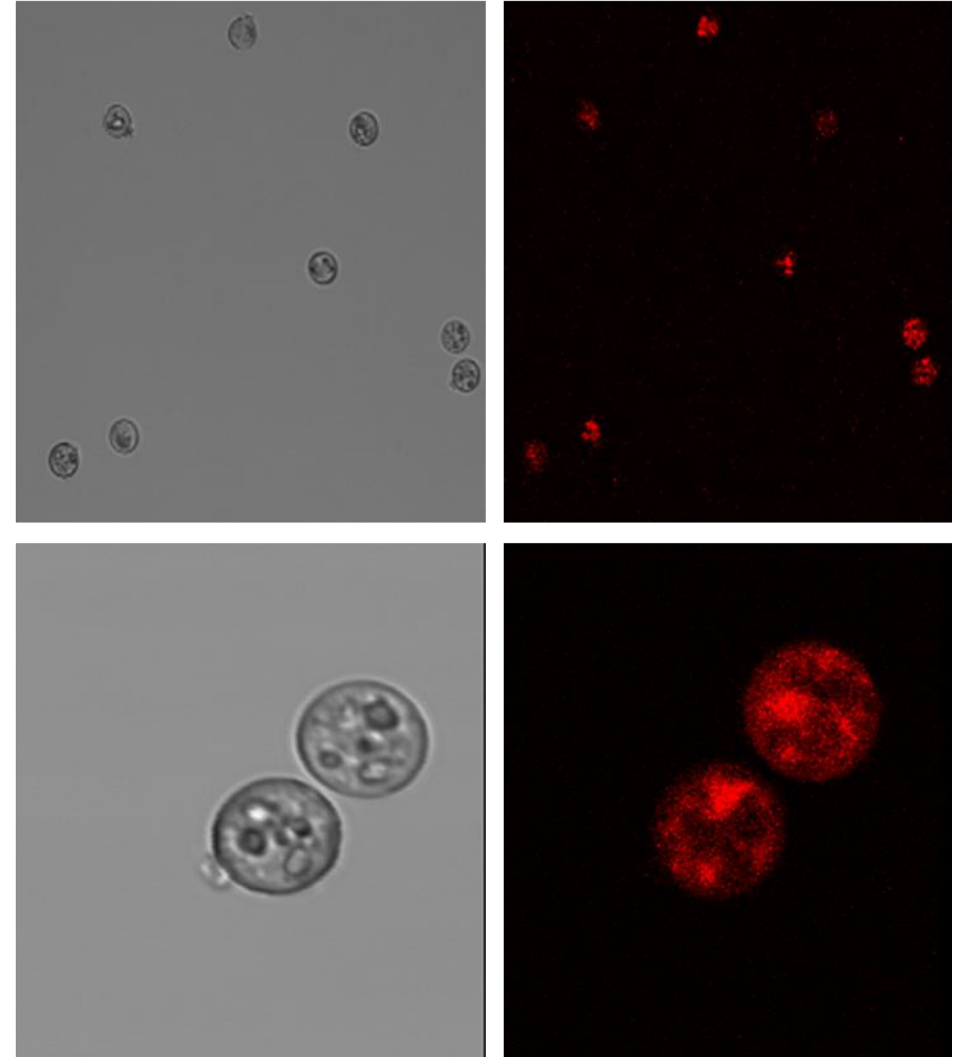


Assay Controls

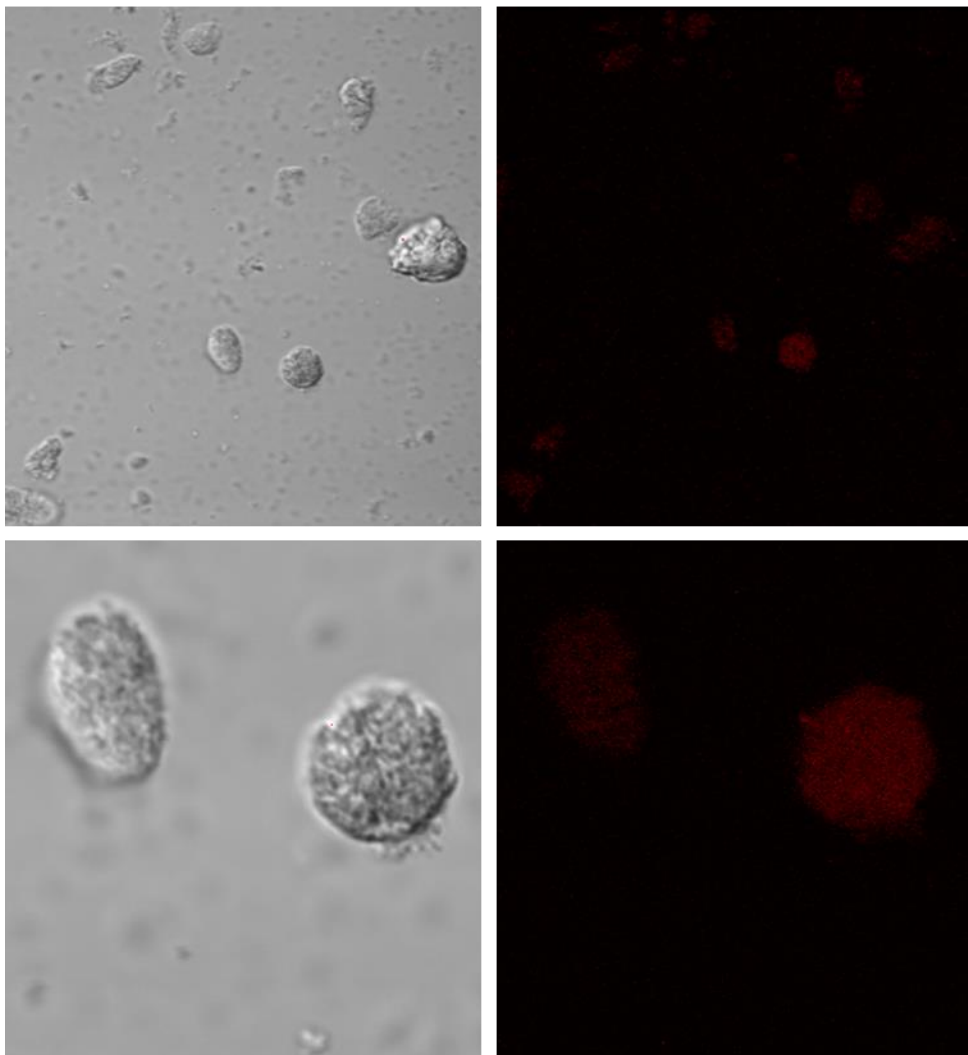
**No Aptamer
(-)ve Control**



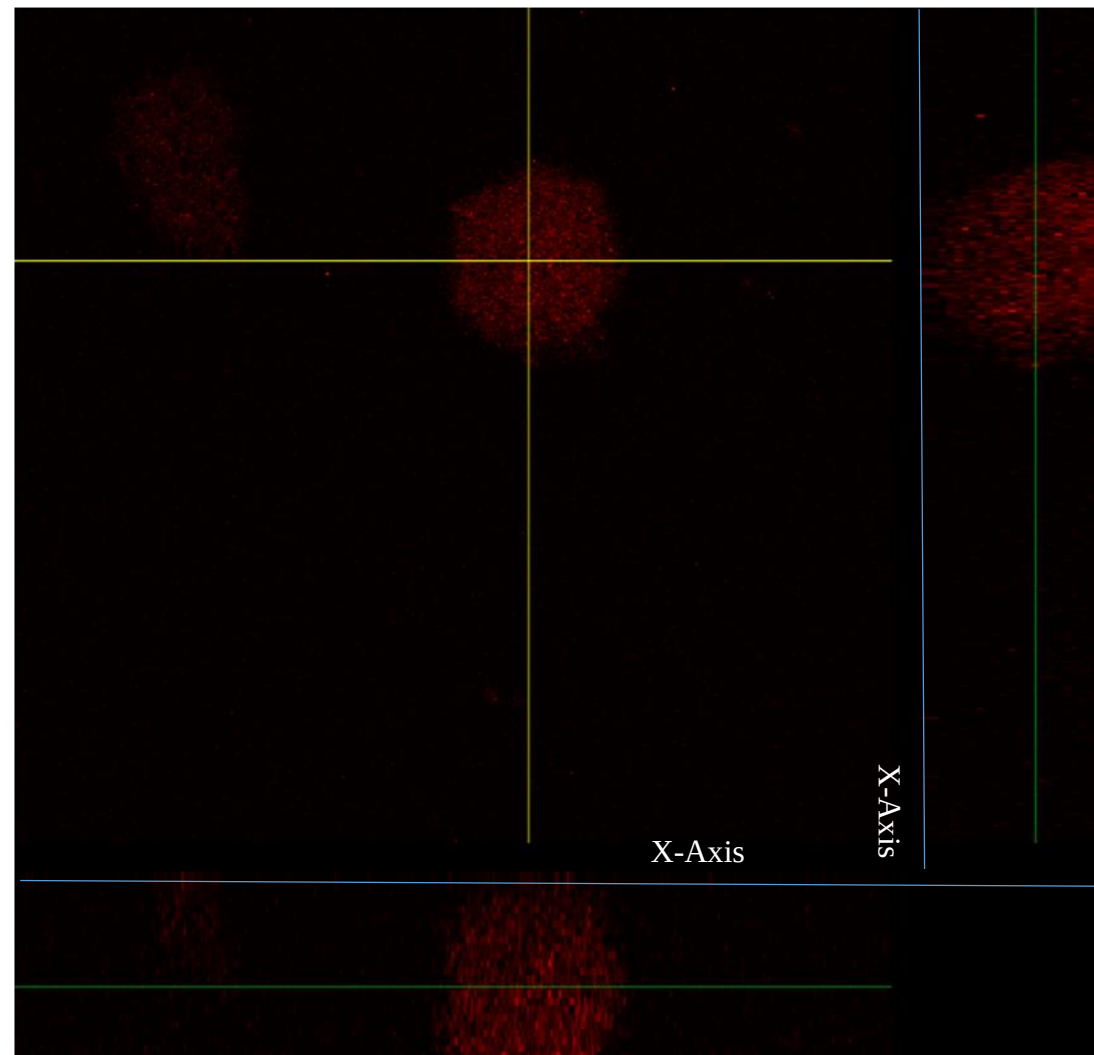
**WP2-1 + Cyclospora
(+)ve Control**



TA4-2



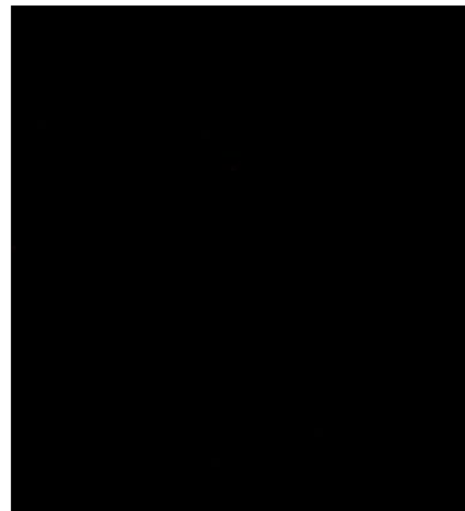
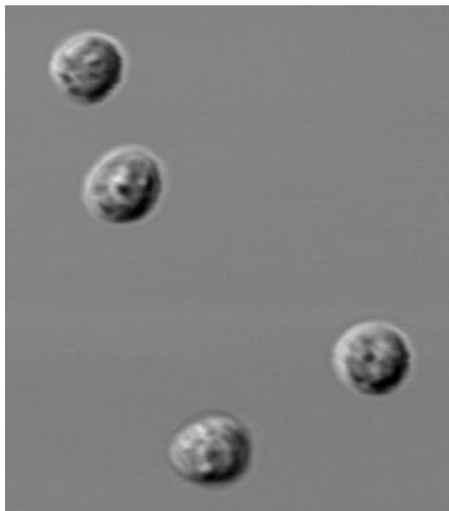
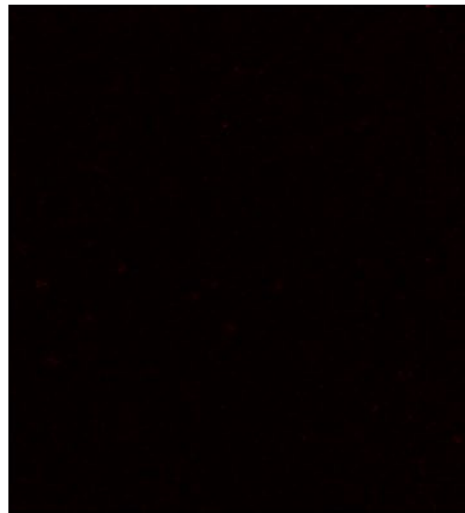
TA4-2 (Z-stack)



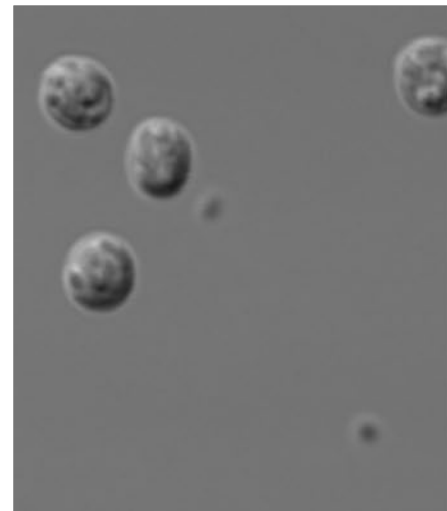
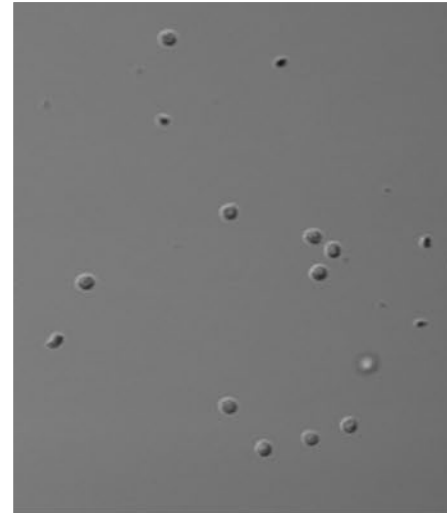
Conclusion: Balamuthia mandurillaris showed weak or insignificant positive signals.

Cryptosporidium Parvum

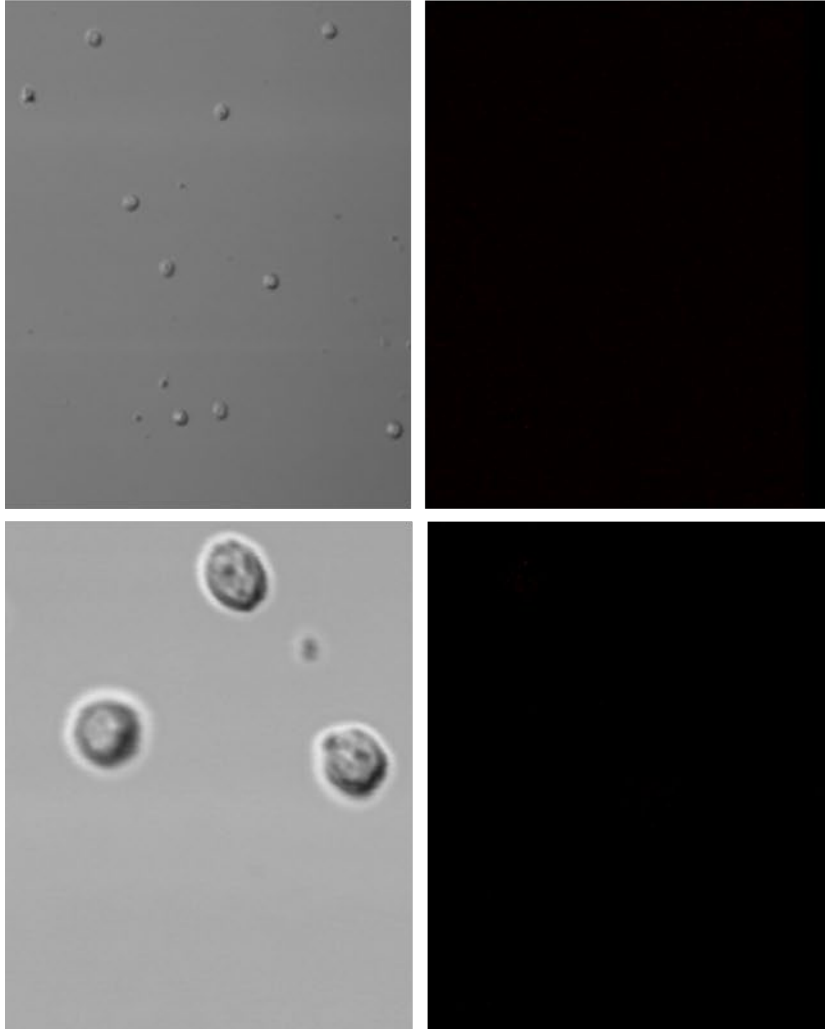
WP2-1



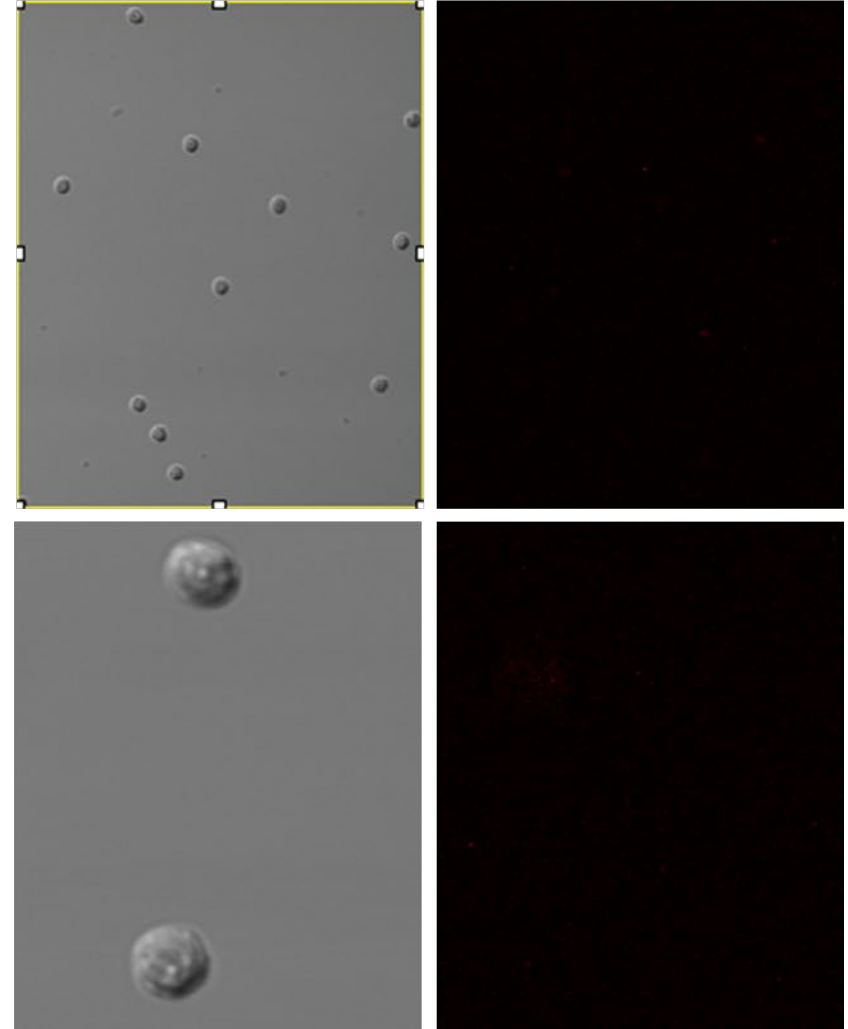
WP2-4



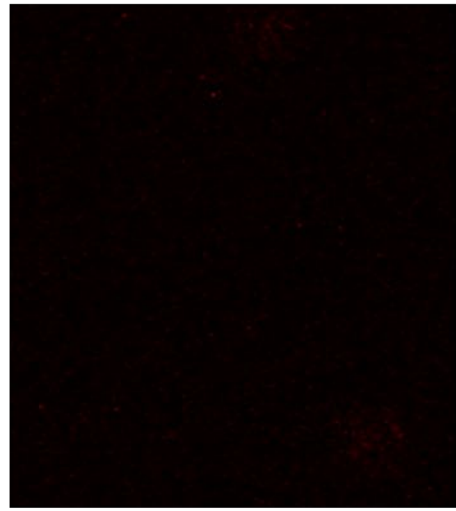
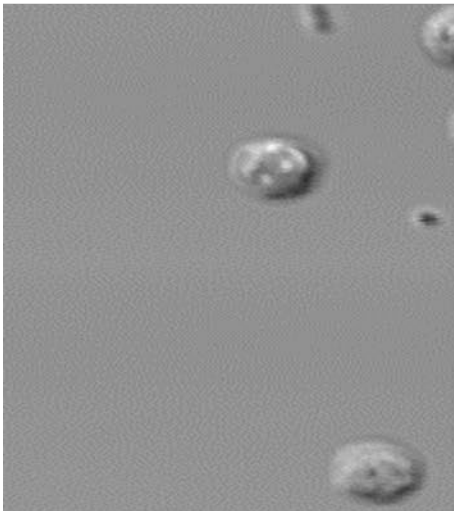
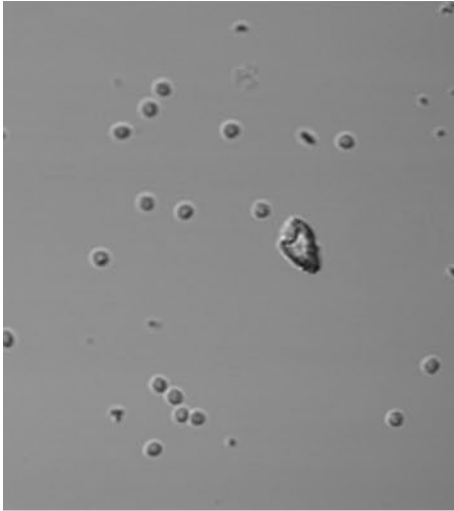
WP2-2



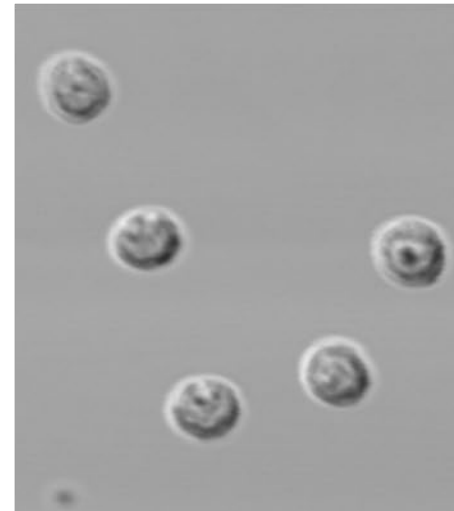
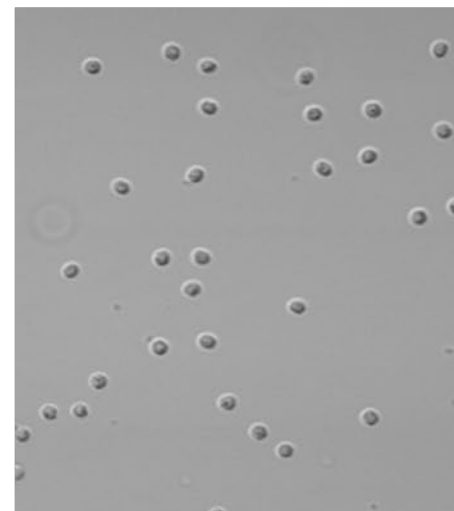
WP2-3



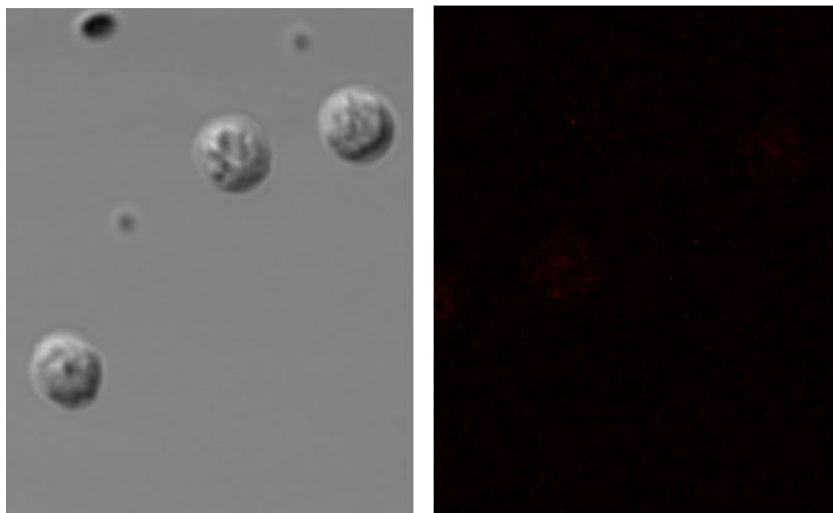
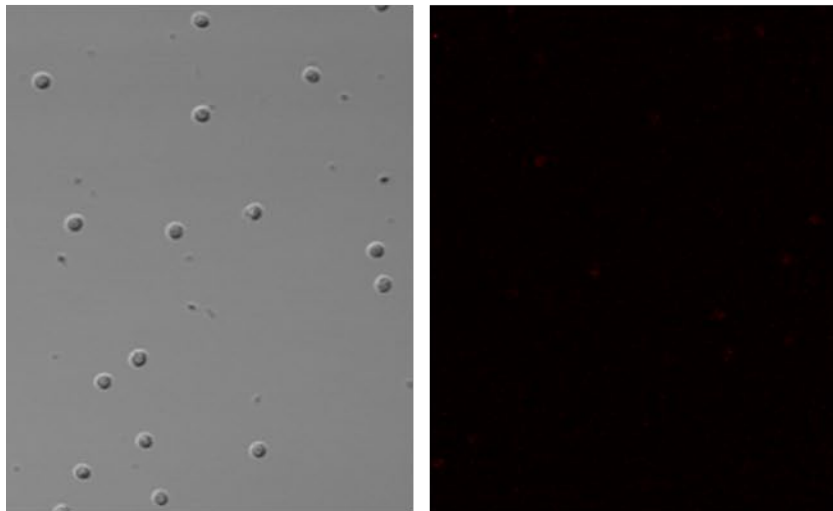
TA4-1



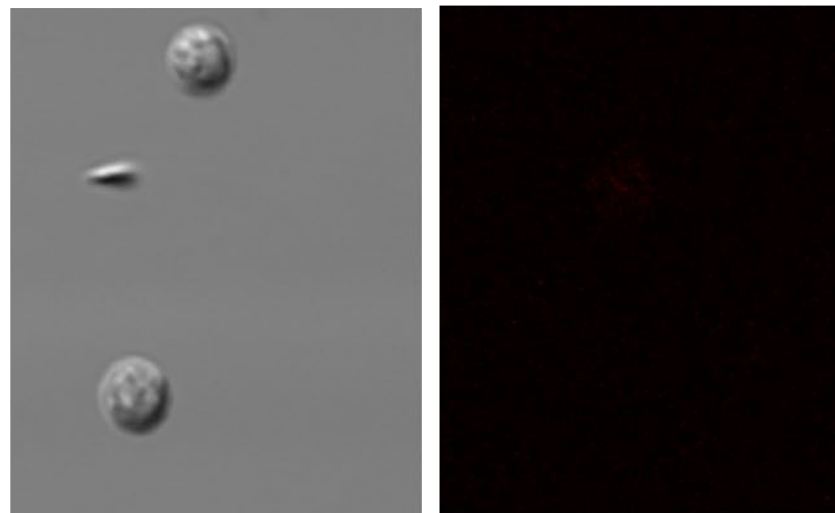
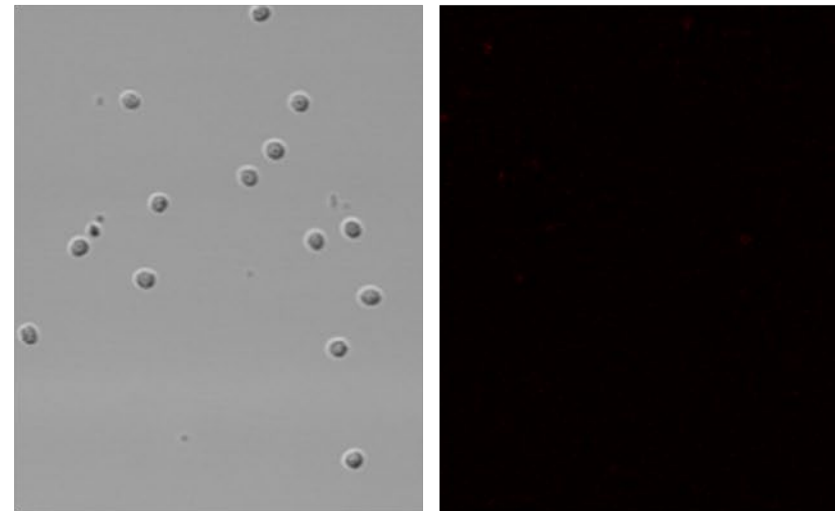
TA4-3



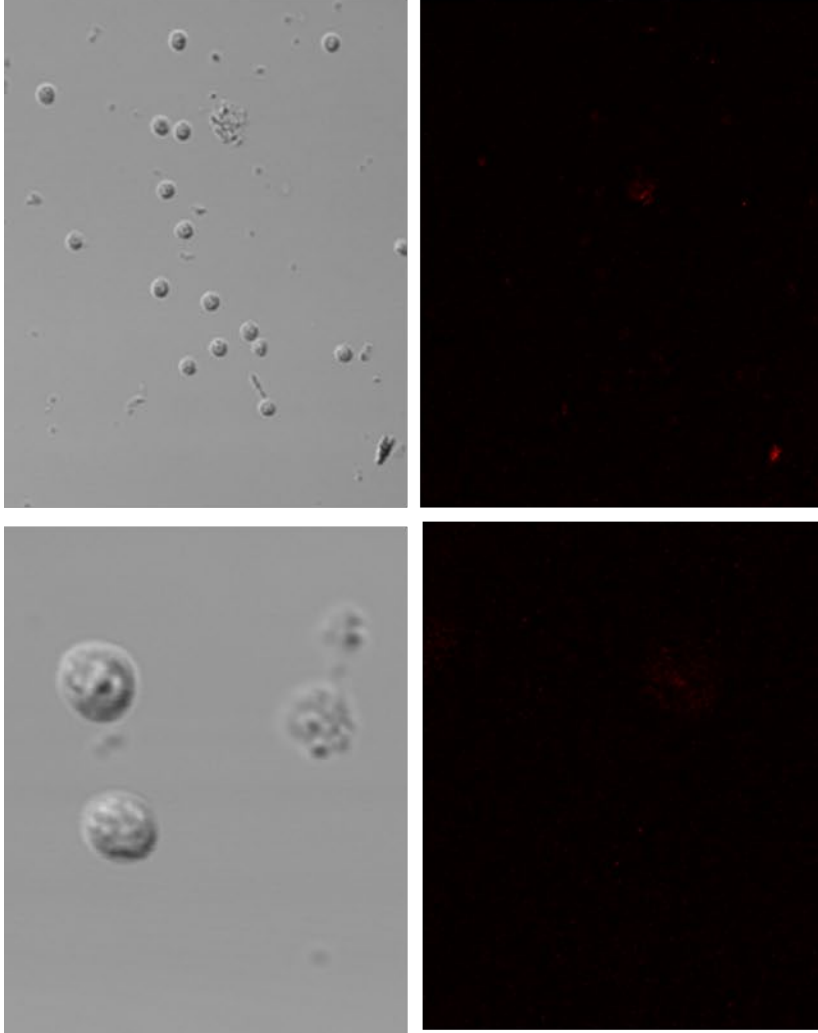
TA4-2



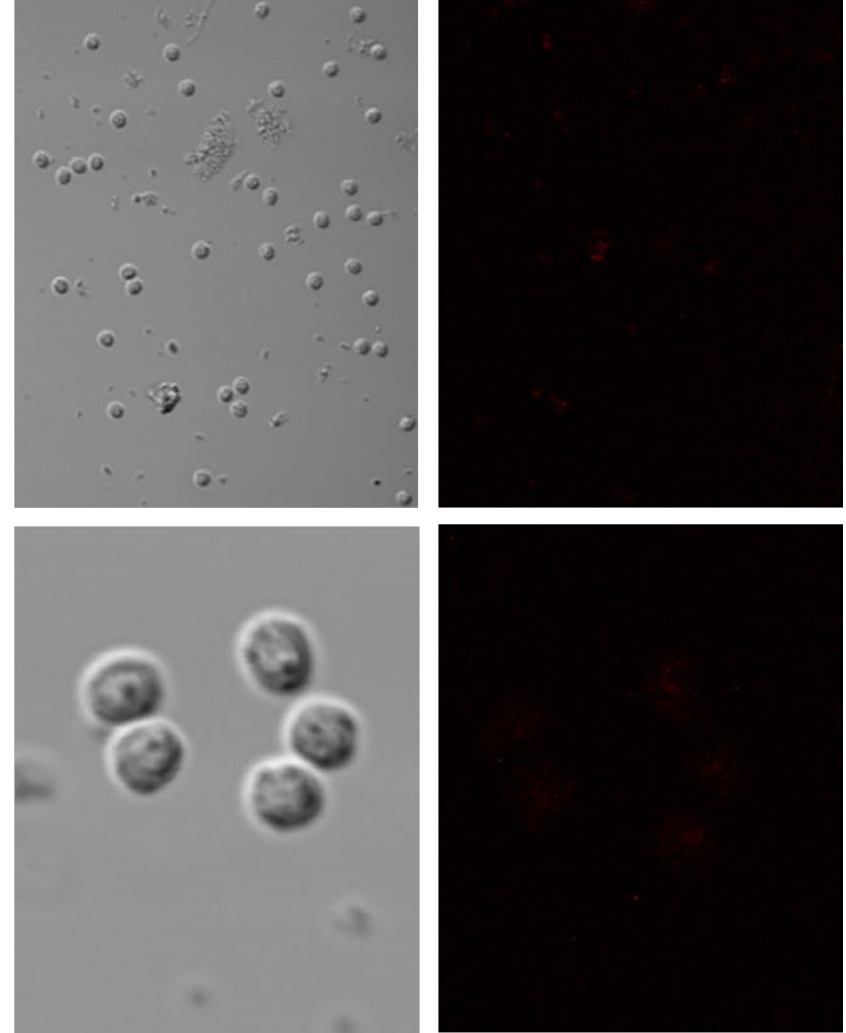
TA4-4



Whole Cyclo S16

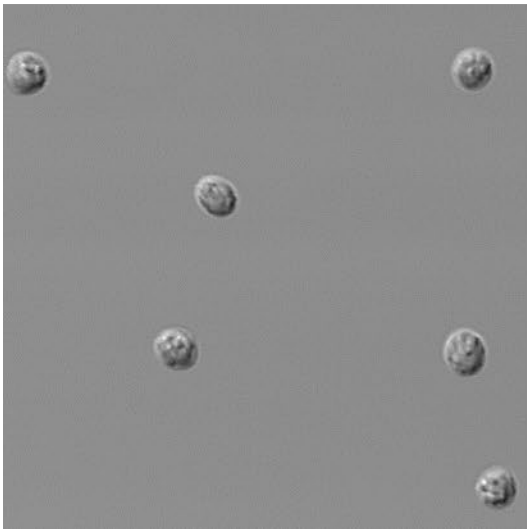
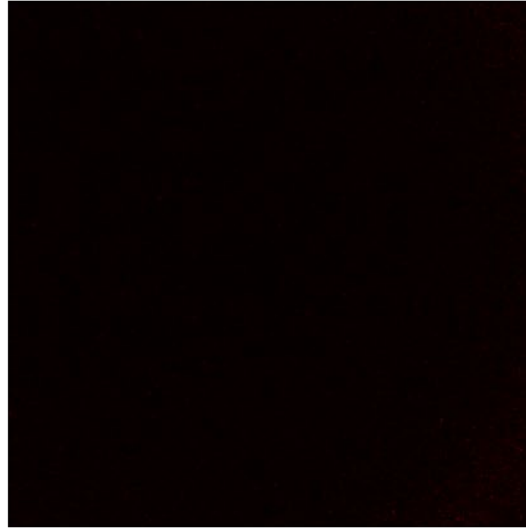
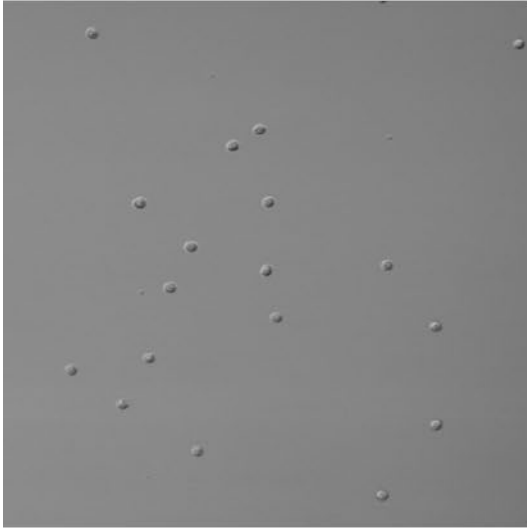


Whole Cyclo S3

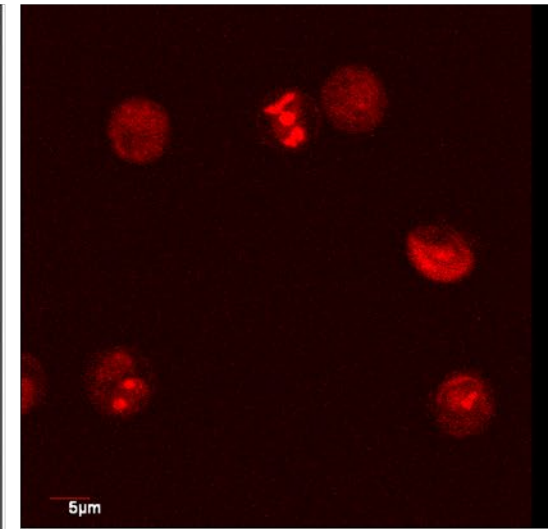
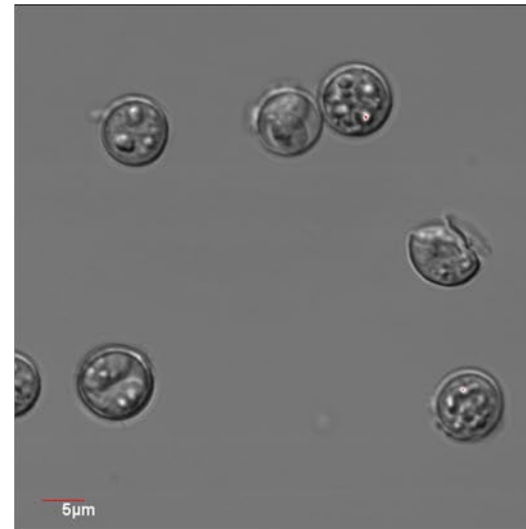
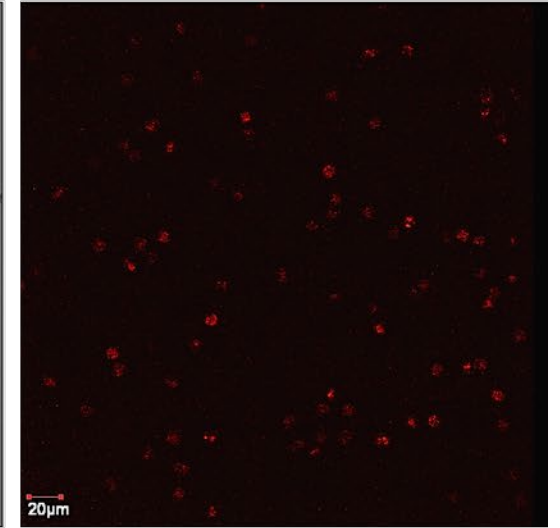
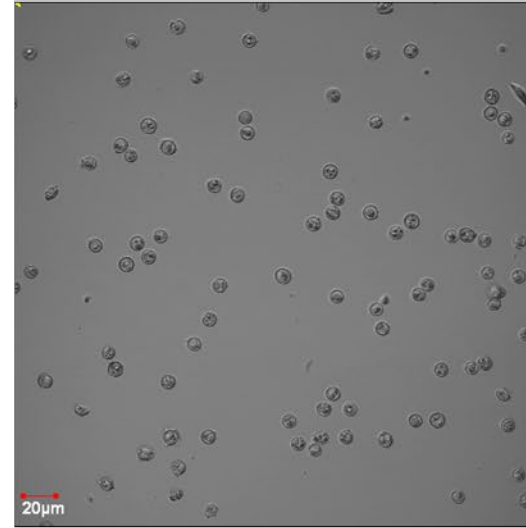


Assay Controls

**No Aptamer
(-)ve Control**



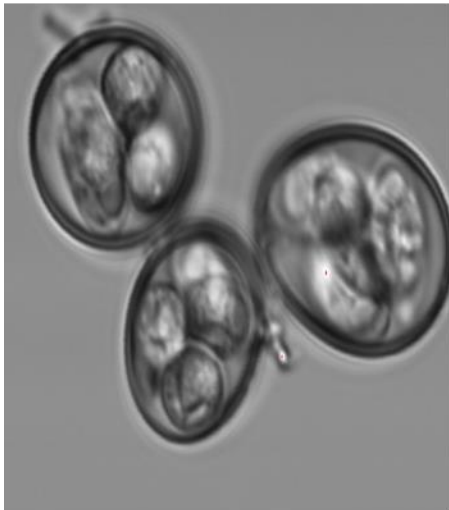
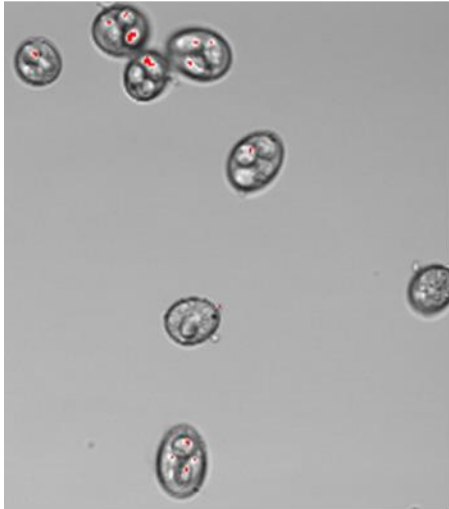
**WP2-1 + Cyclospora
(+)ve Control**



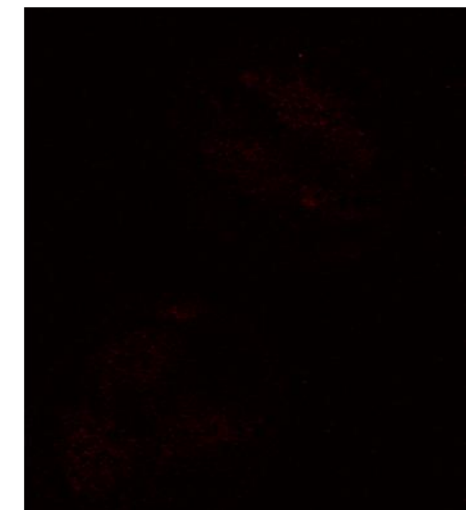
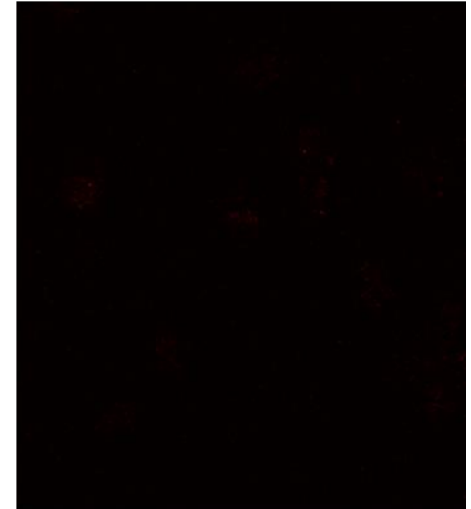
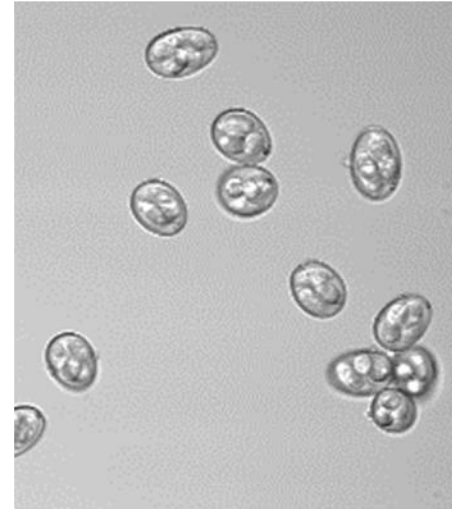
Conclusion: *Cryptosporidium parvum* showed no positive signals to any of the aptamer

Eimaria Acervulina

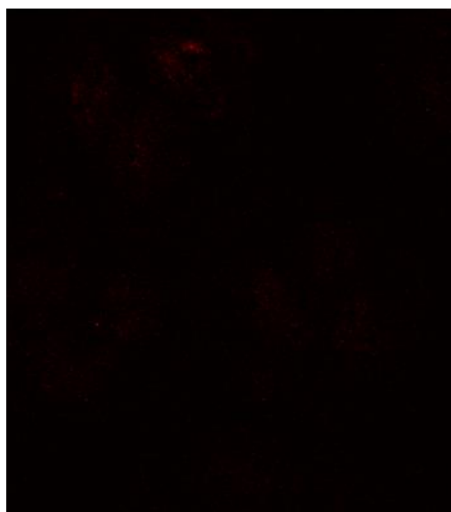
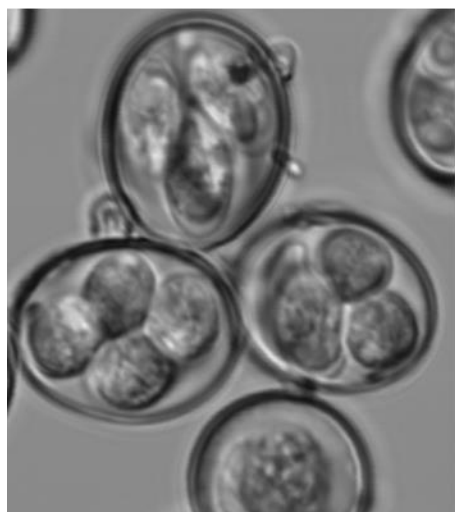
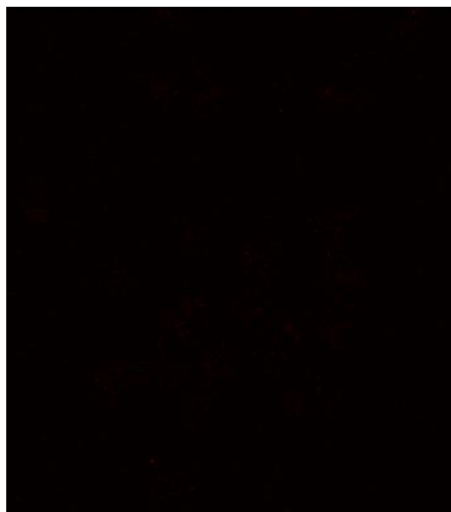
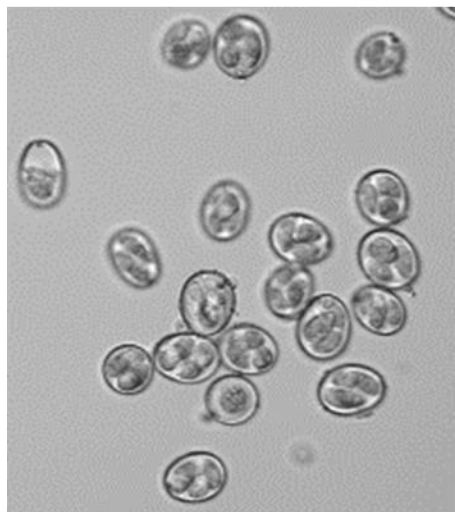
WP2-1



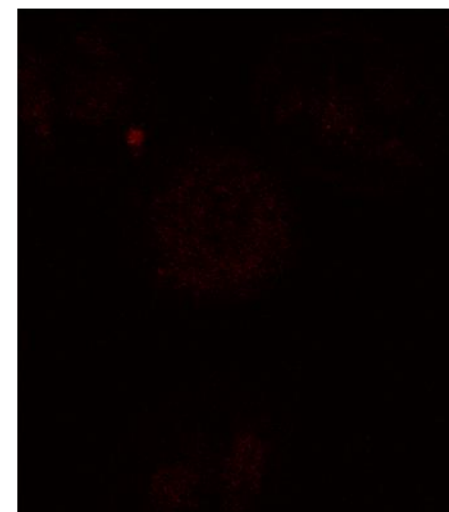
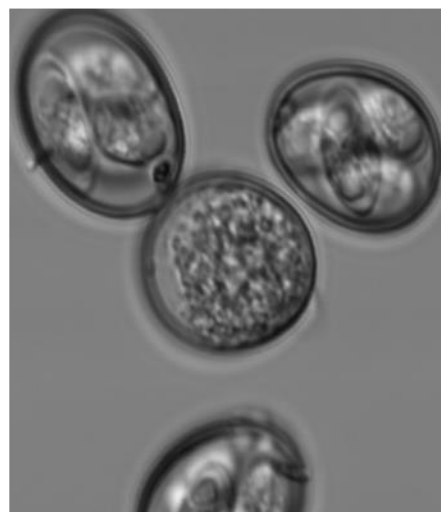
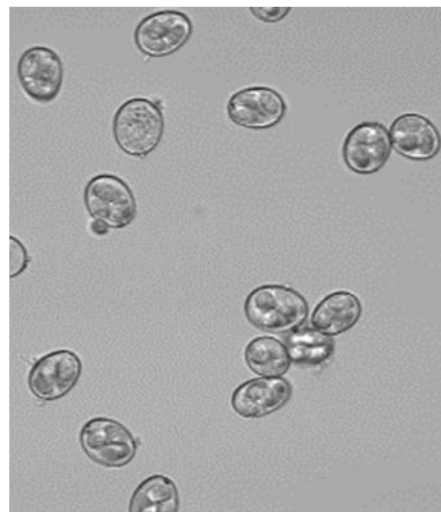
WP2-4



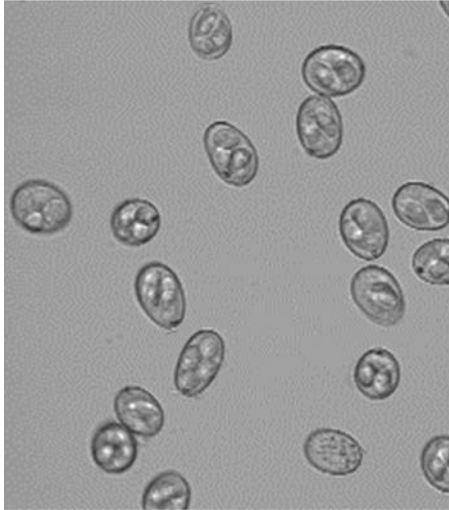
WP2-2



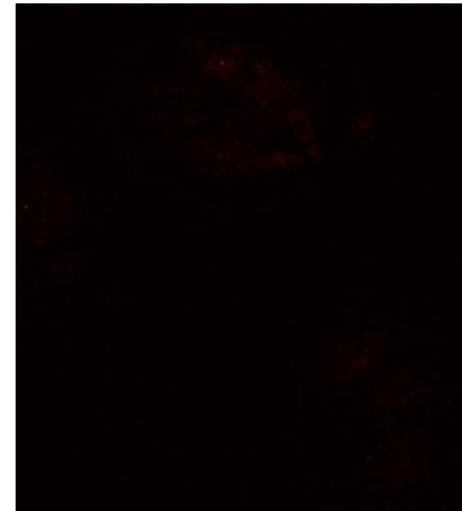
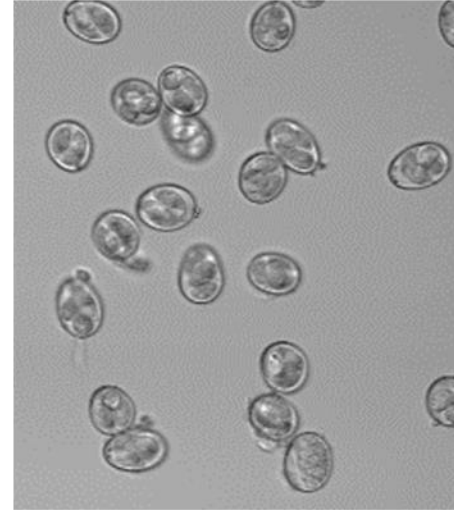
WP2-3



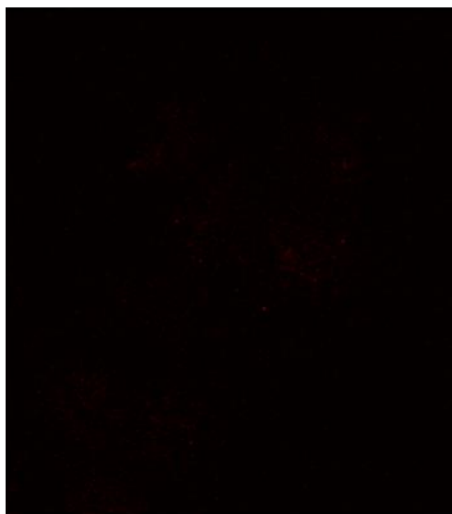
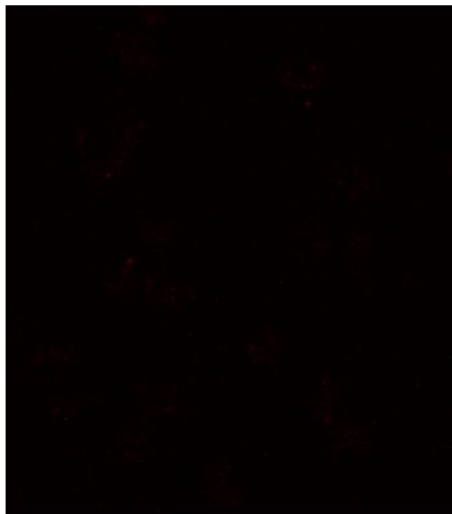
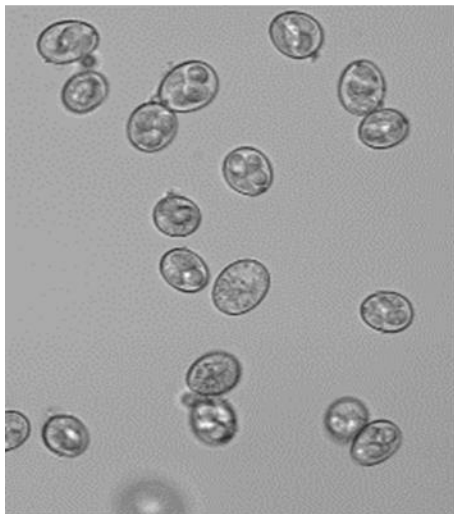
TA4-1



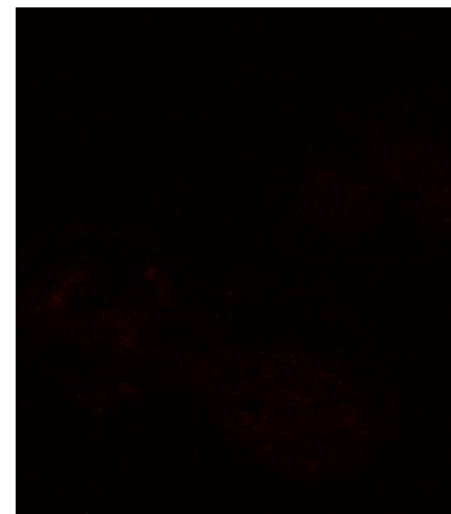
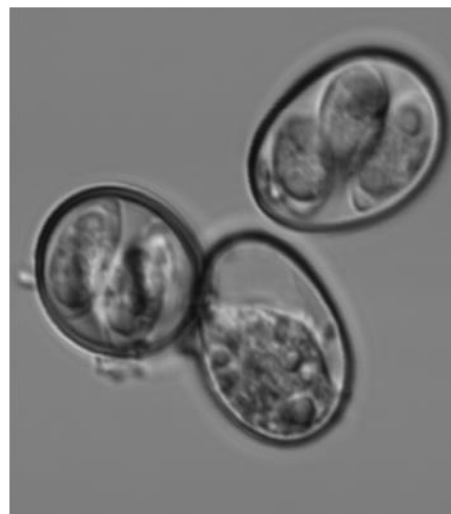
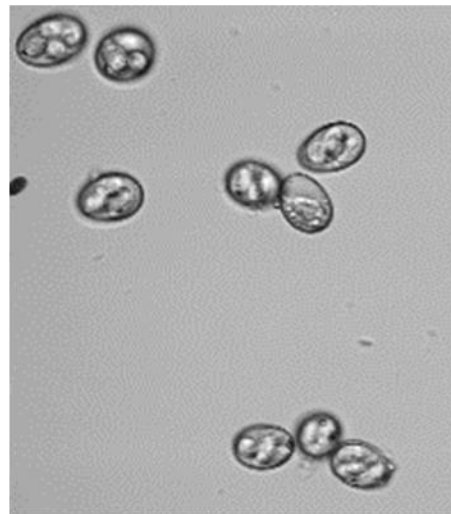
TA4-3



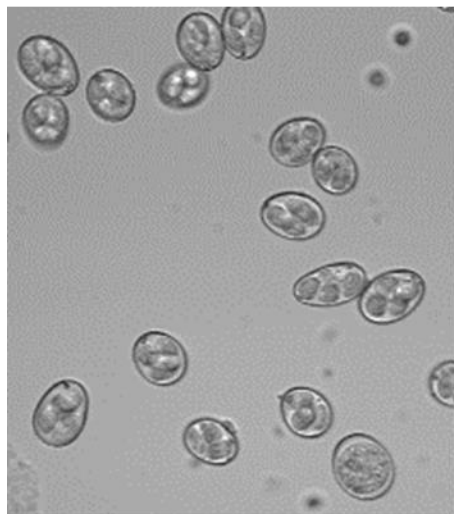
TA4-2



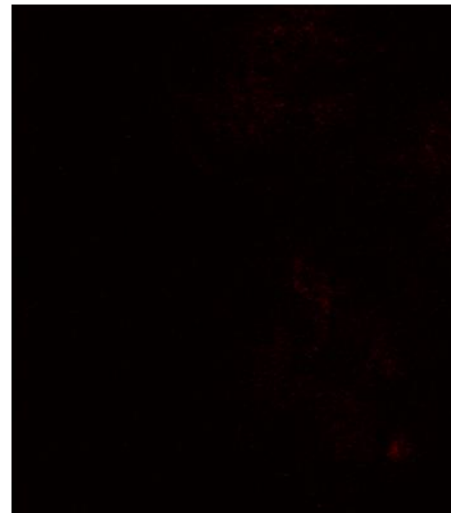
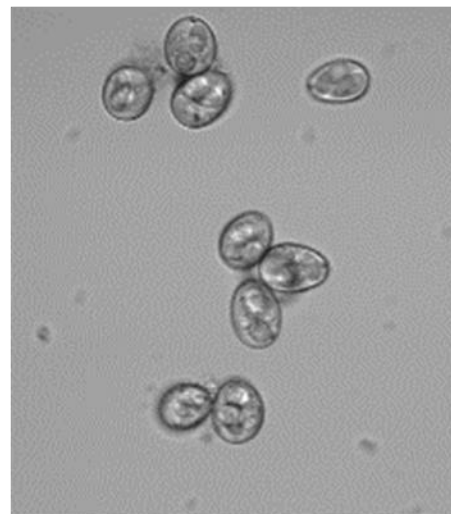
TA4-4



Whole Cyclo S16

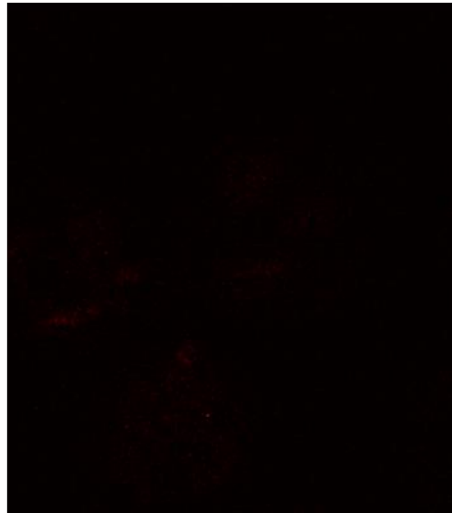
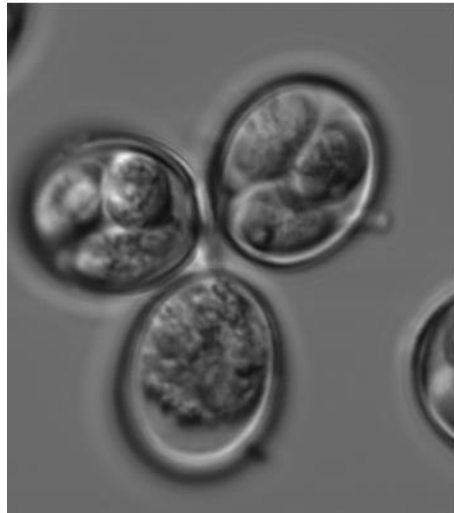
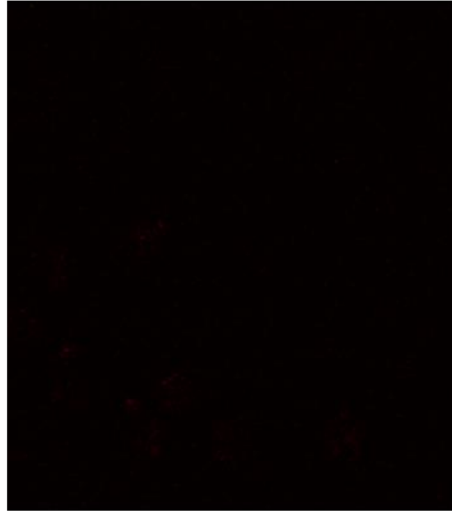
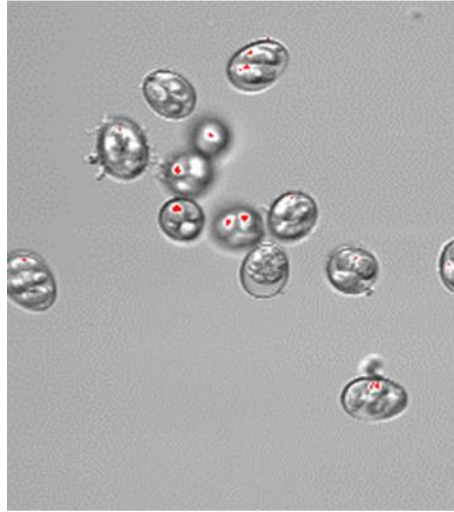


Whole Cyclo S3

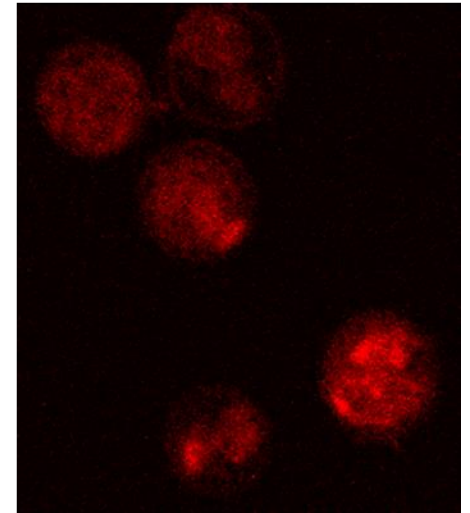
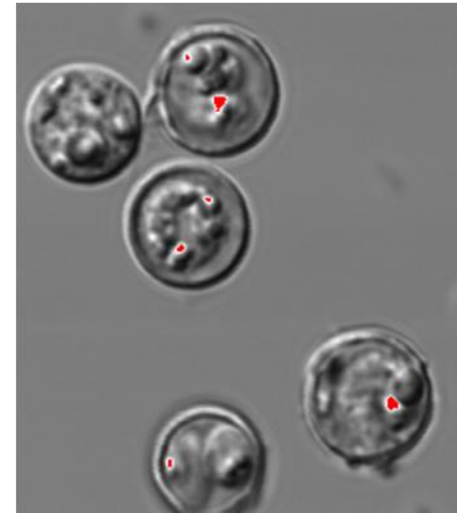
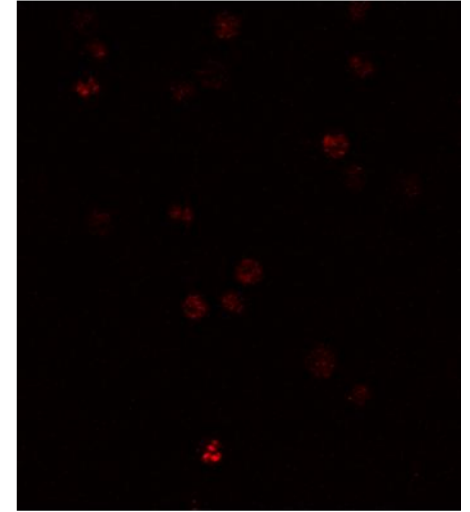
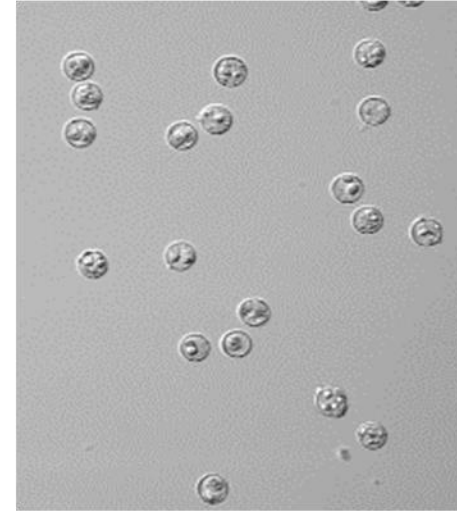


Assay Controls

**No Aptamer
(-)ve Control**



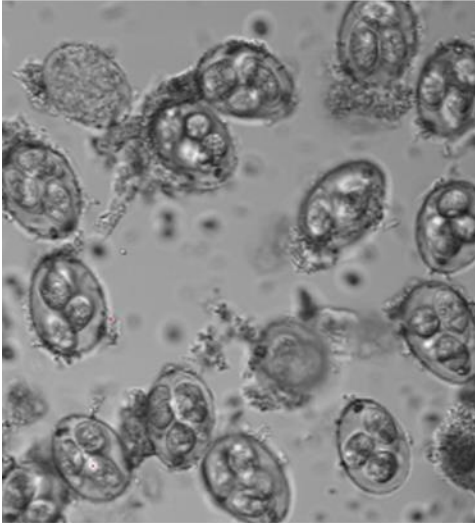
**WP2-1 + Cyclospora
(+)ve Control**



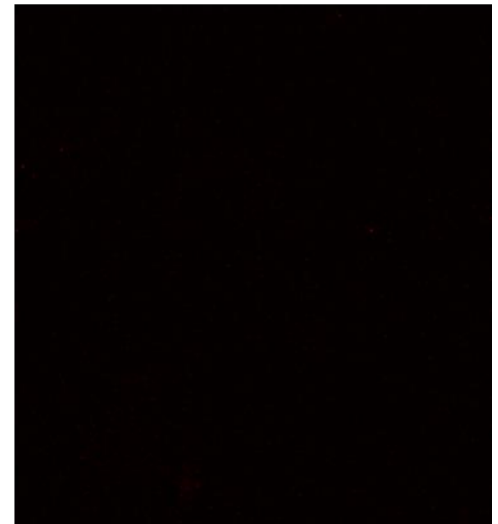
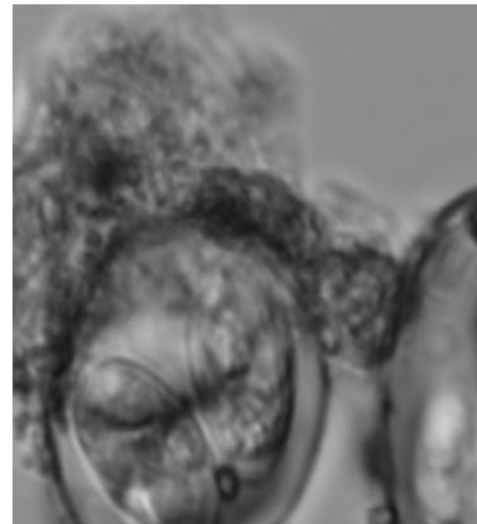
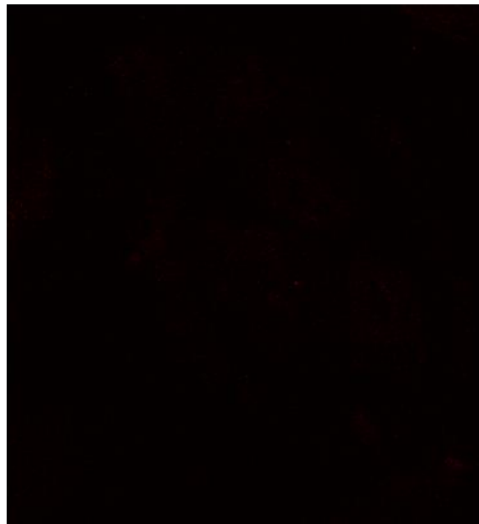
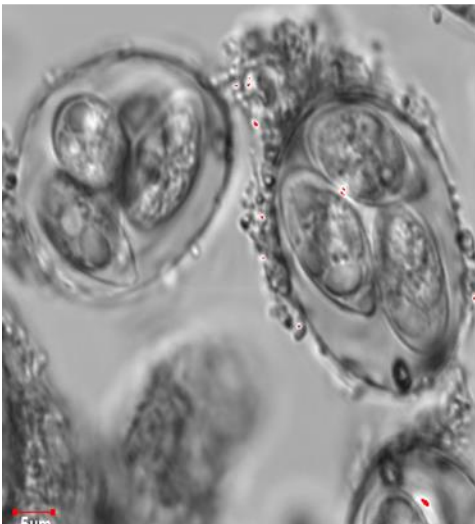
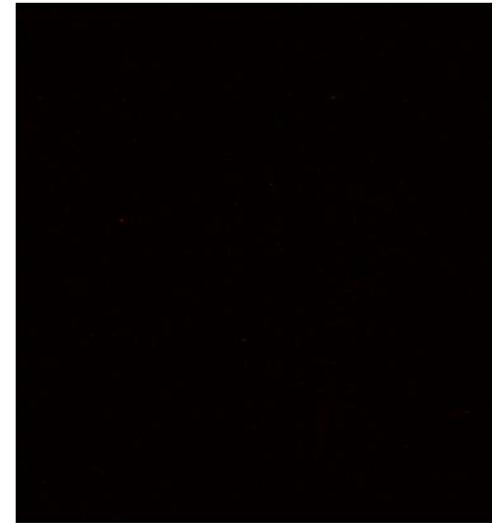
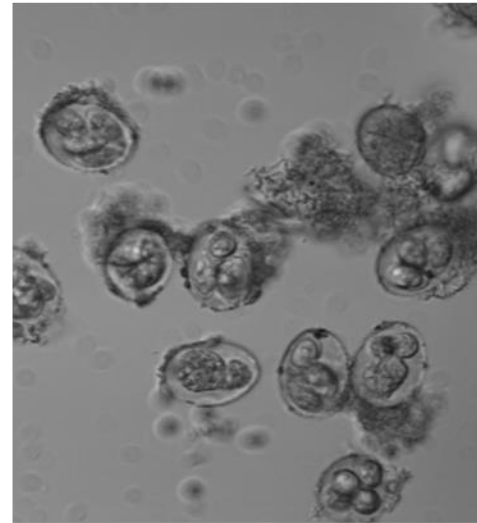
Conclusion: *Eimaria acervulina* showed no positive signals with any of the aptamers.

Eimaria Maxima

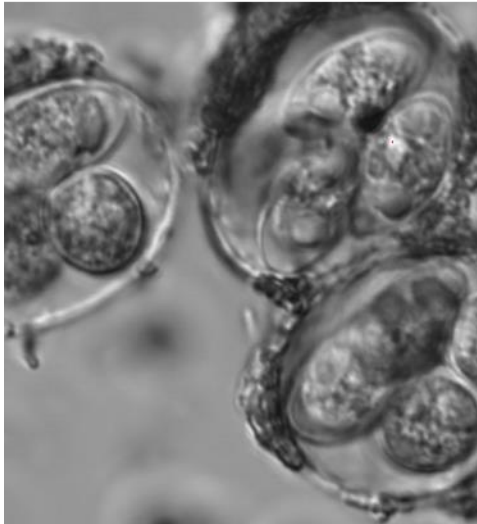
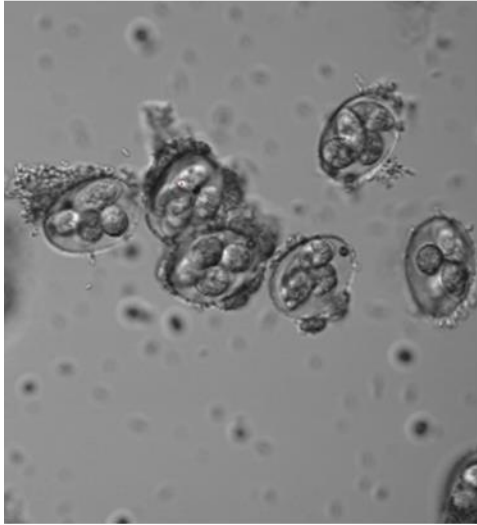
WP2-1



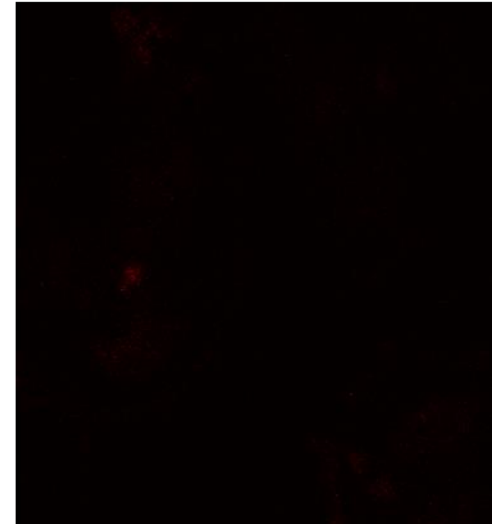
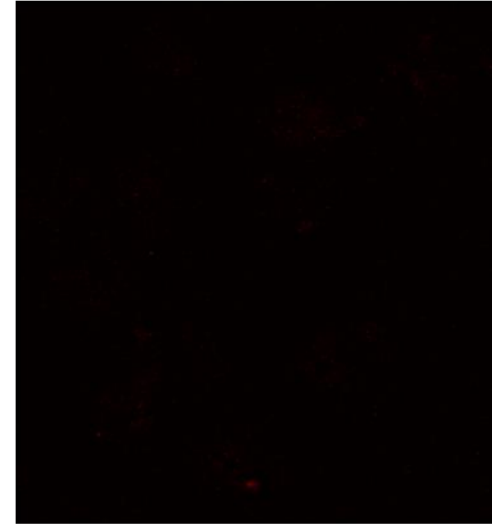
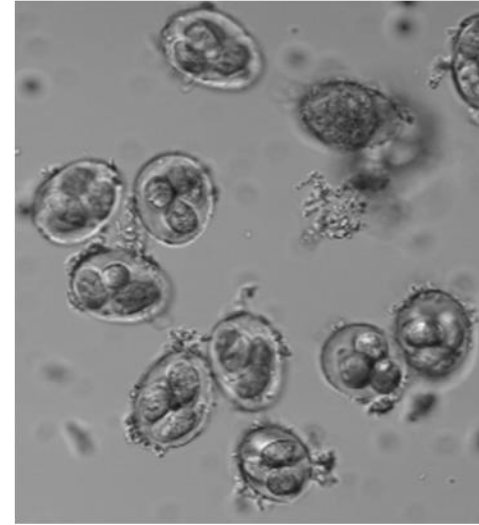
WP2-4



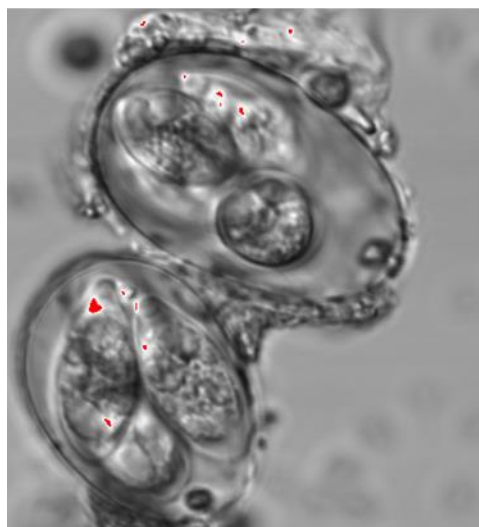
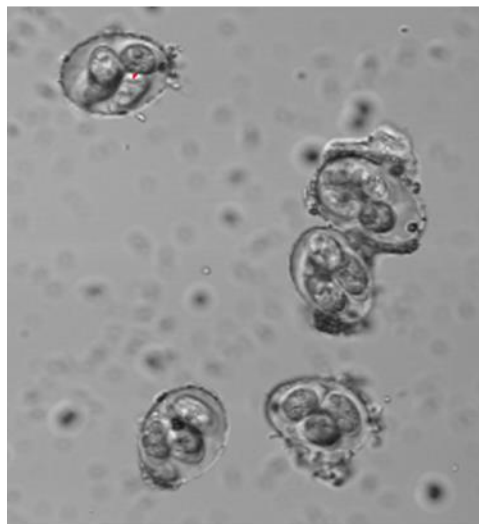
WP2-2



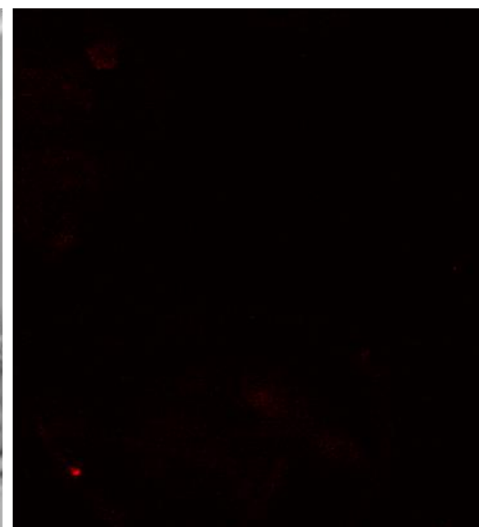
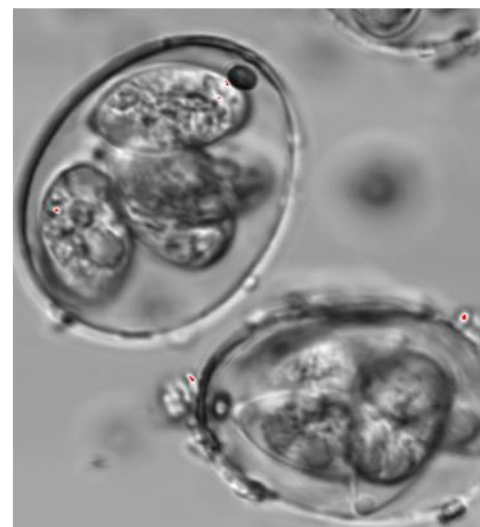
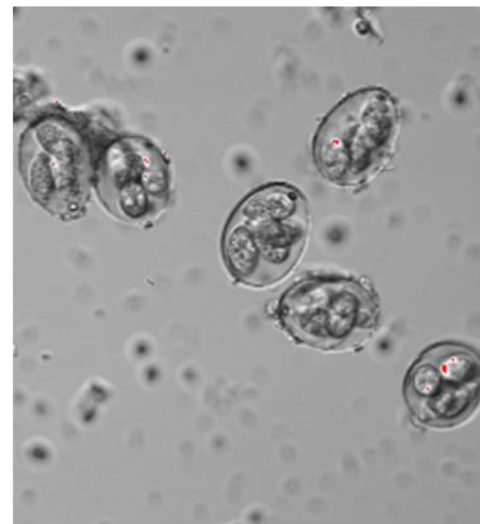
WP2-3



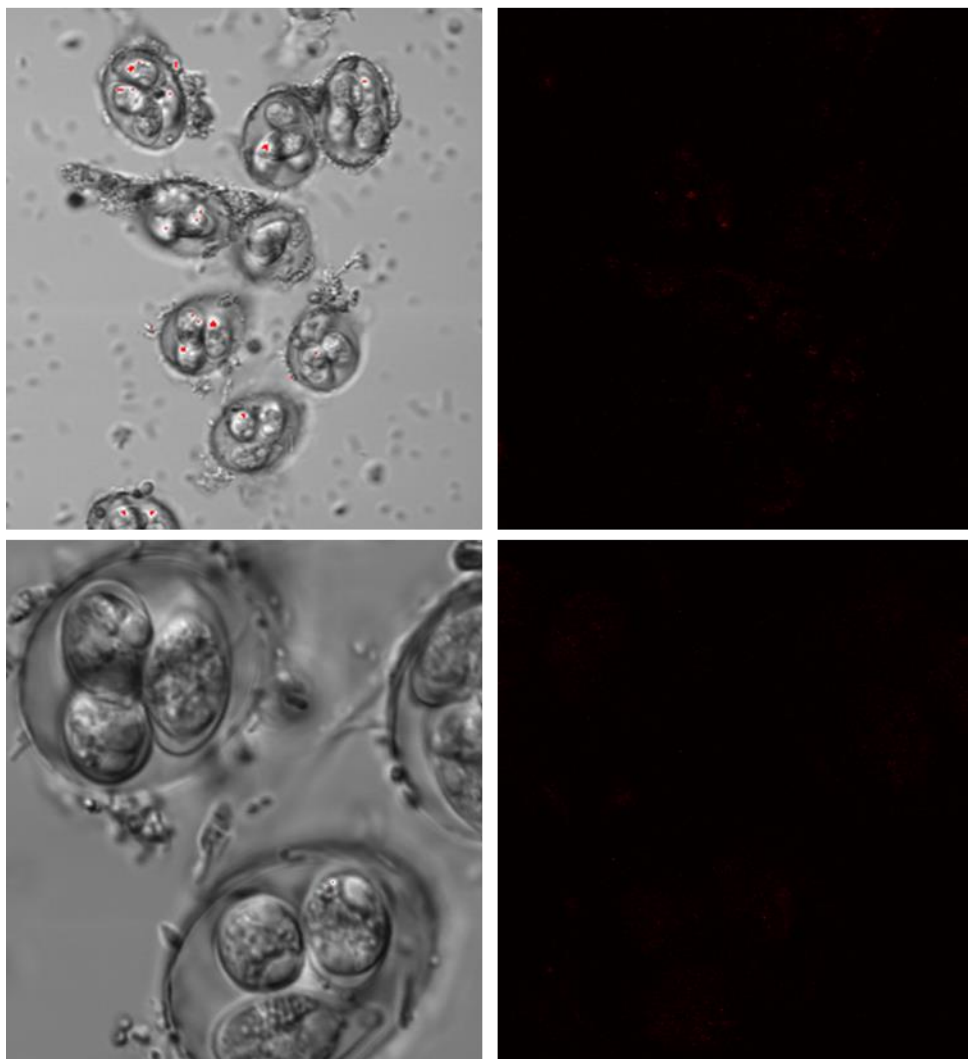
TA4-1



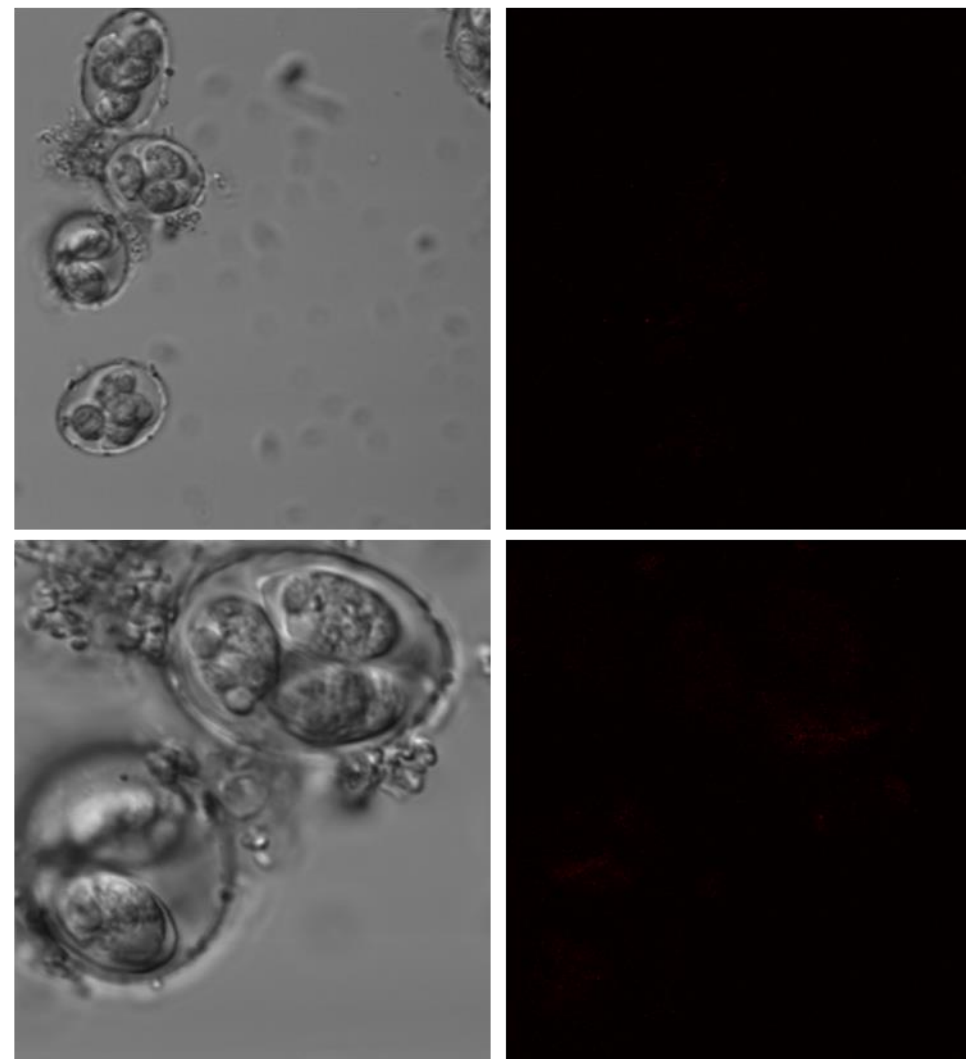
TA4-3



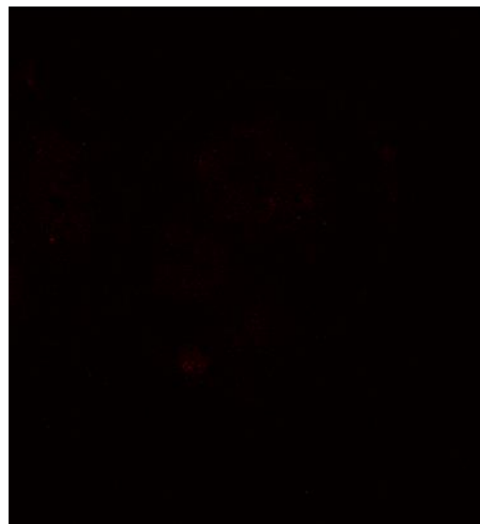
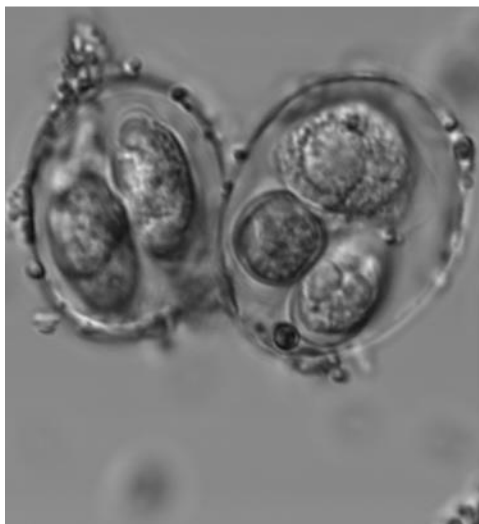
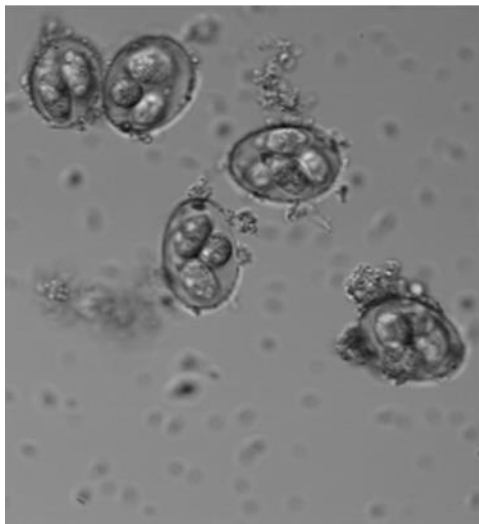
TA4-2



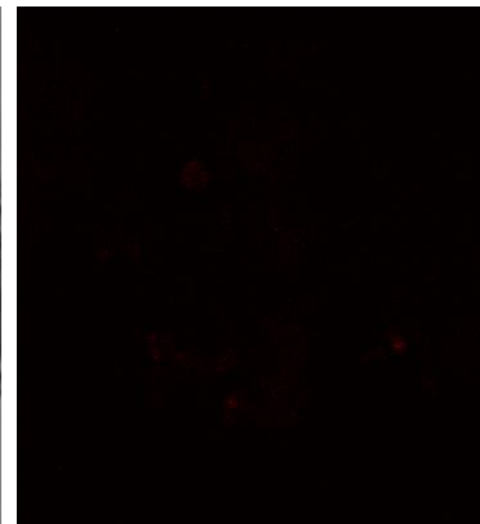
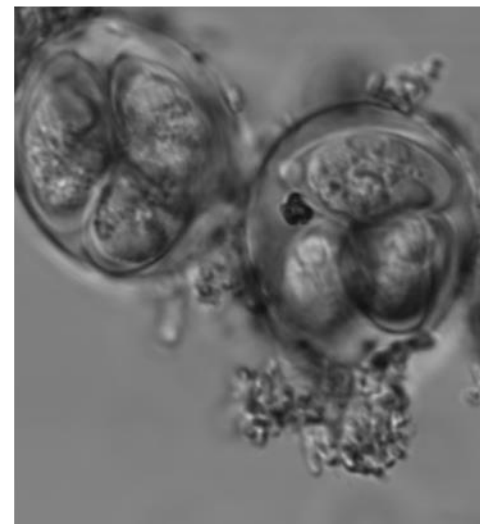
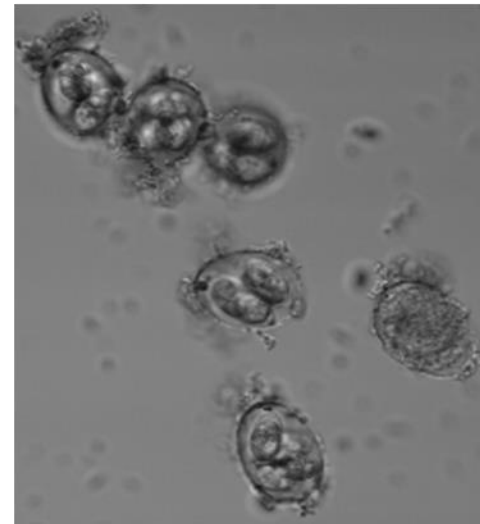
TA4-4



Whole Cyclo S16

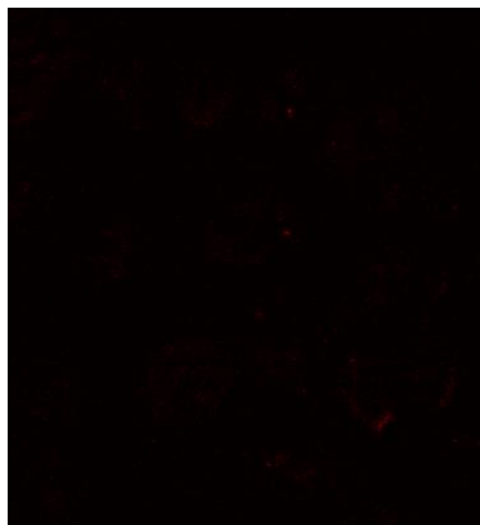
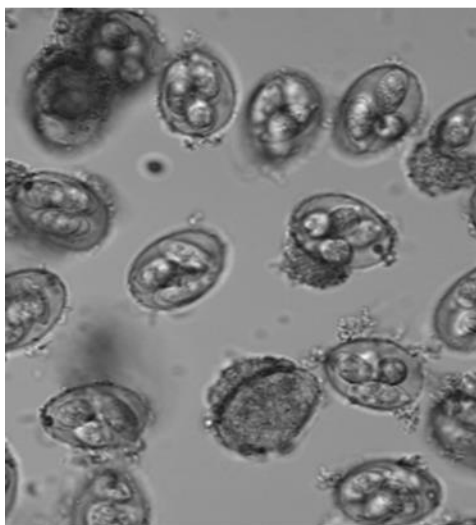


Whole Cyclo S3

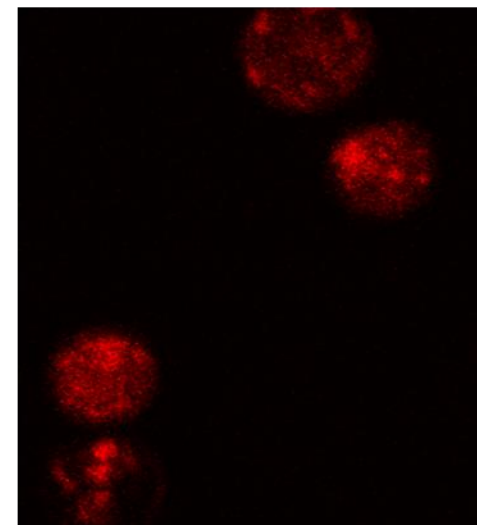
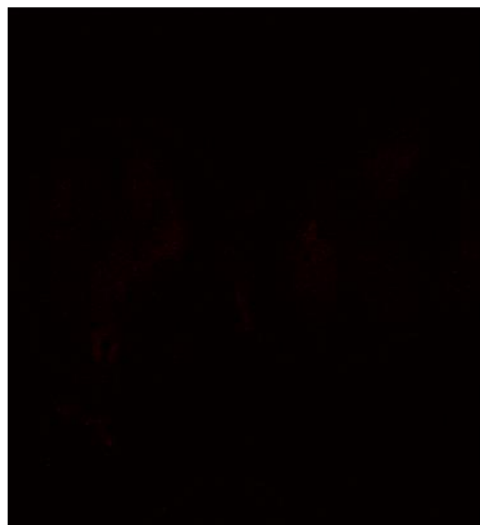
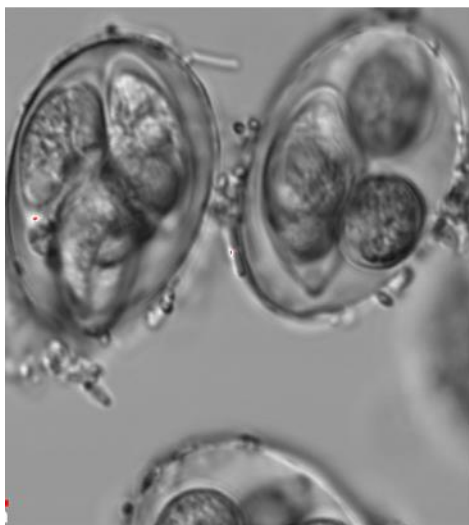
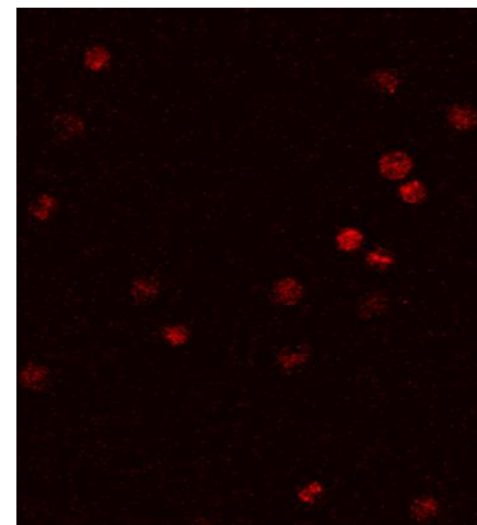
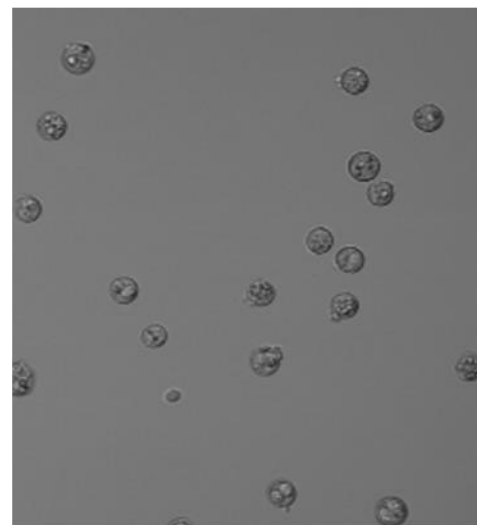


Assay Controls

**No Aptamer
(-)ve Control**



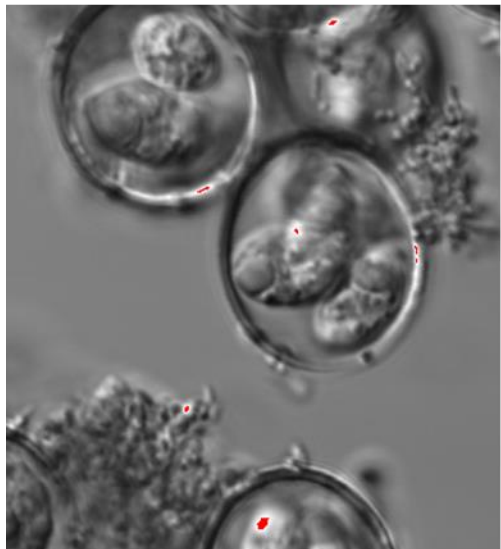
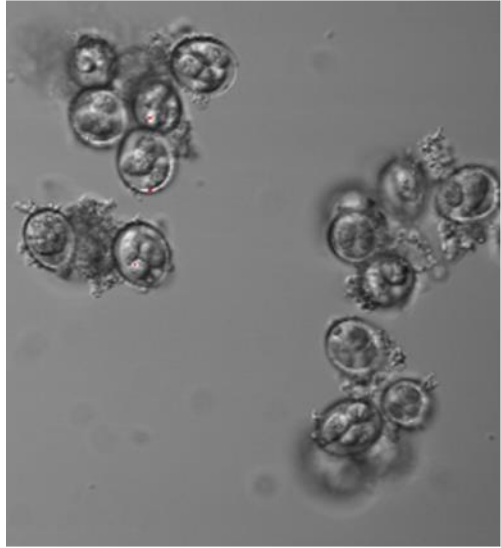
**WP2-1 + Cyclospora
(+)ve Control**



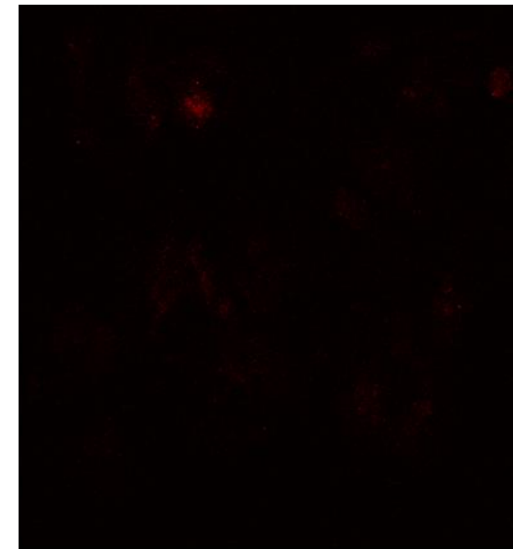
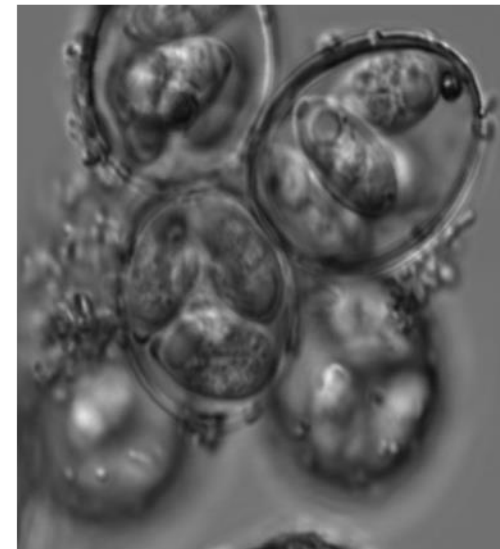
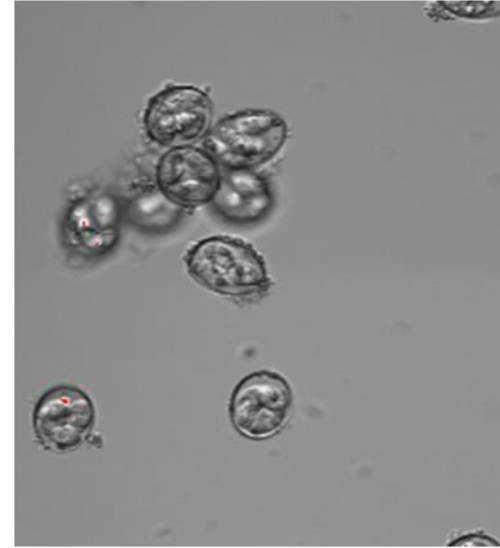
Conclusion: *Eimeria maxima* showed no positive signals with any of the aptamers.

Eimaria Tenella

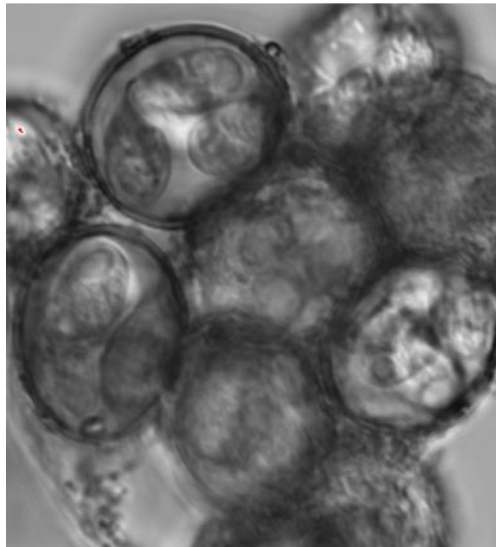
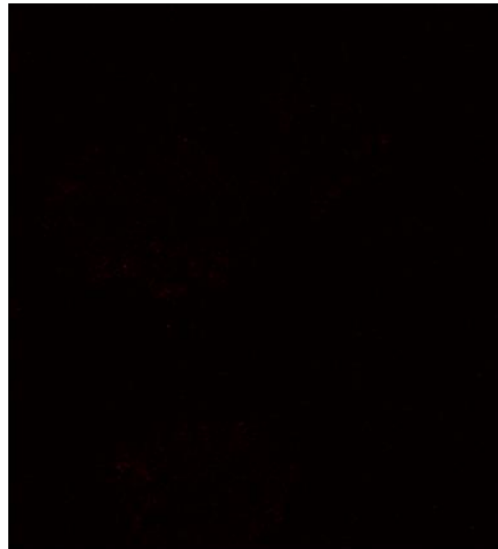
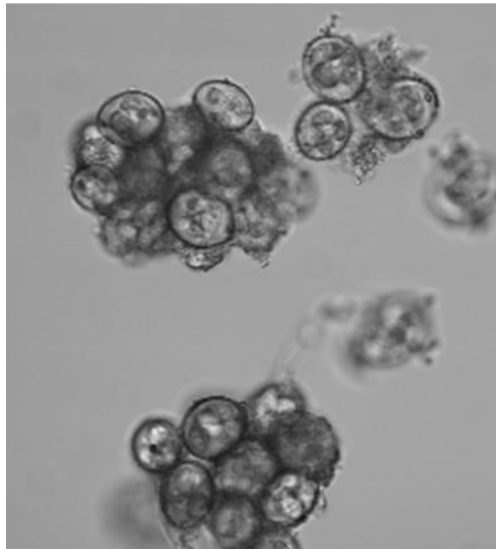
WP2-1



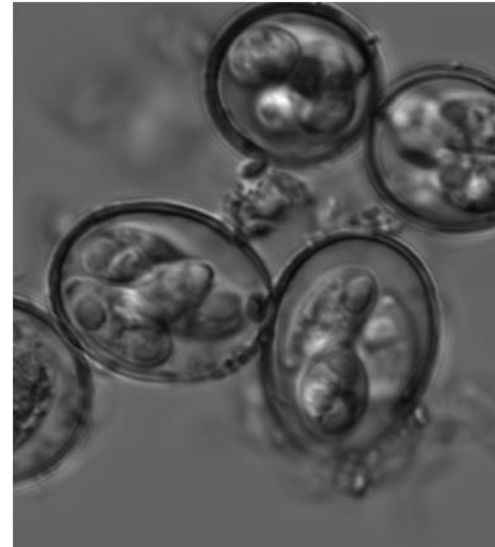
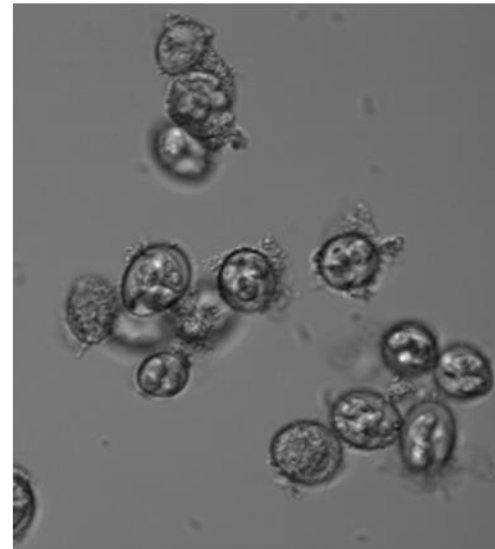
WP2-4



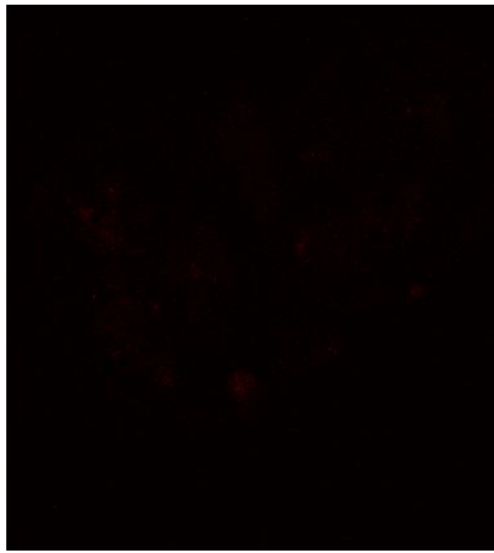
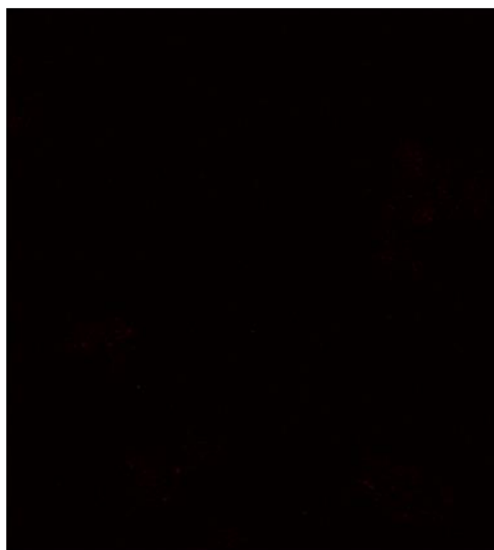
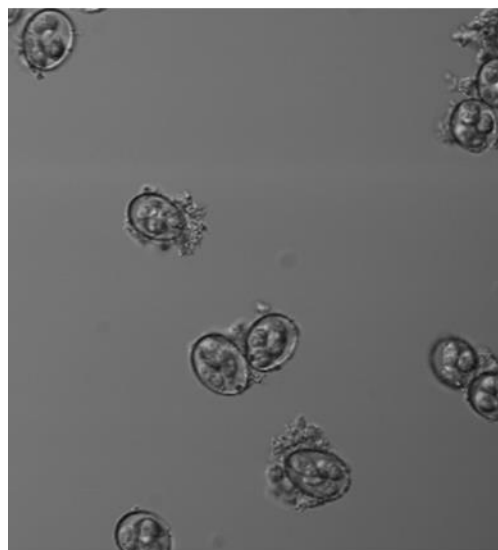
WP2-2



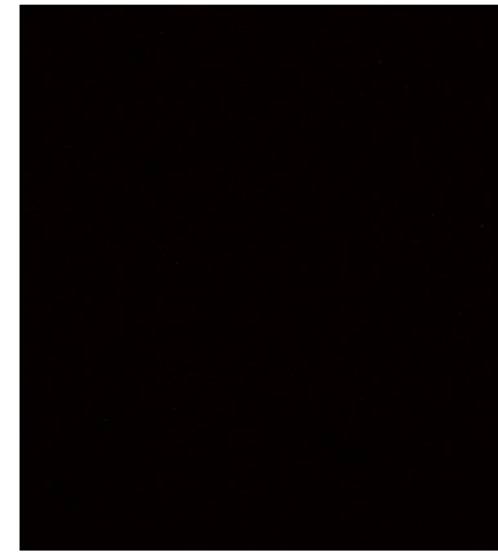
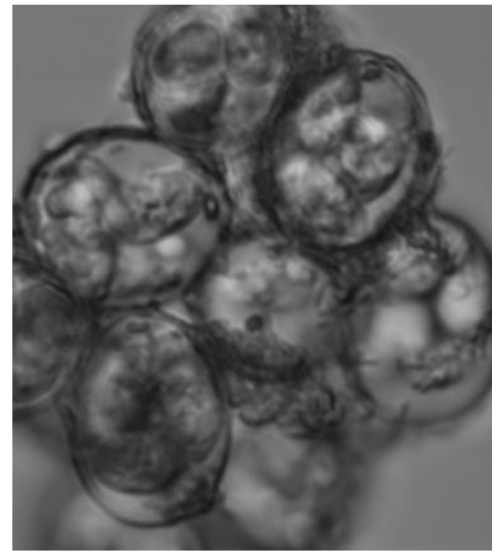
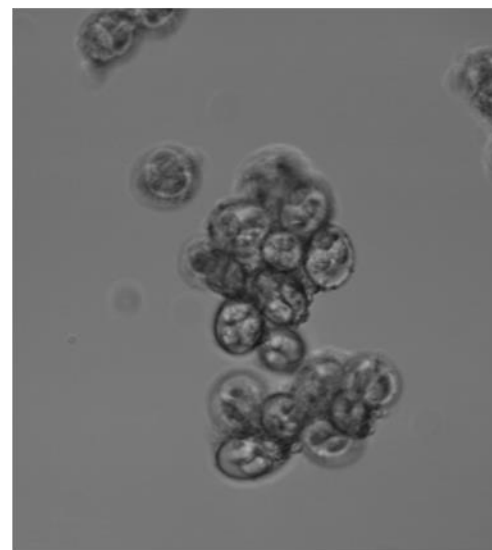
WP2-3



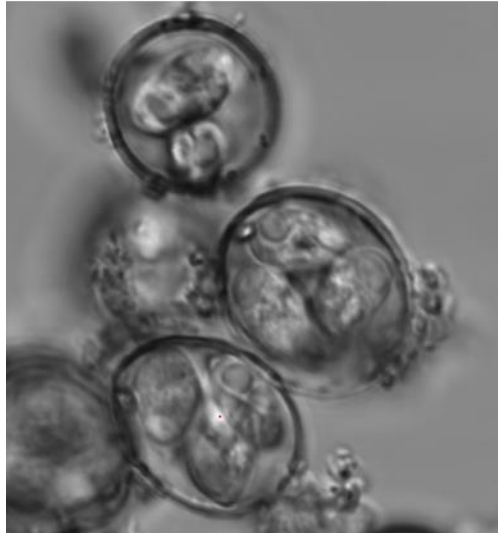
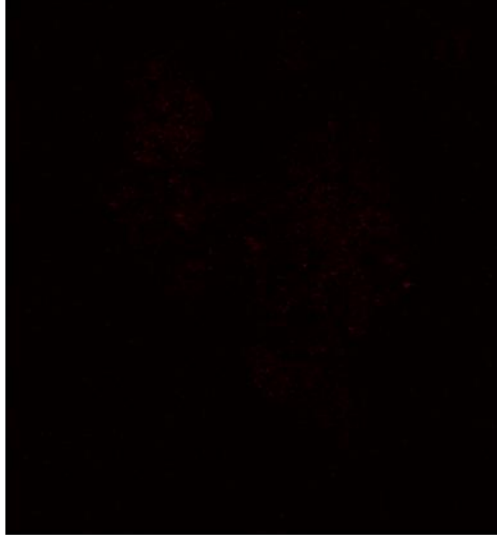
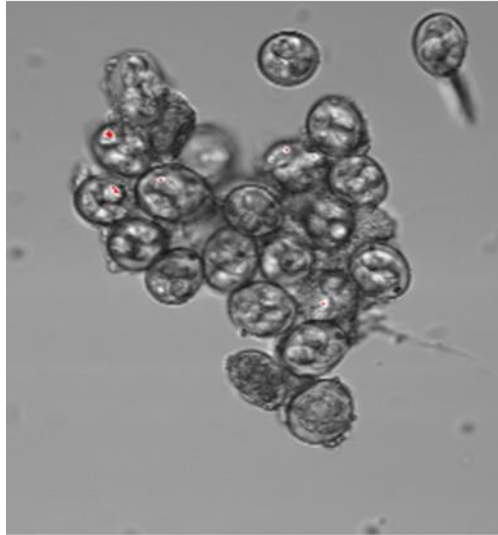
TA4-1



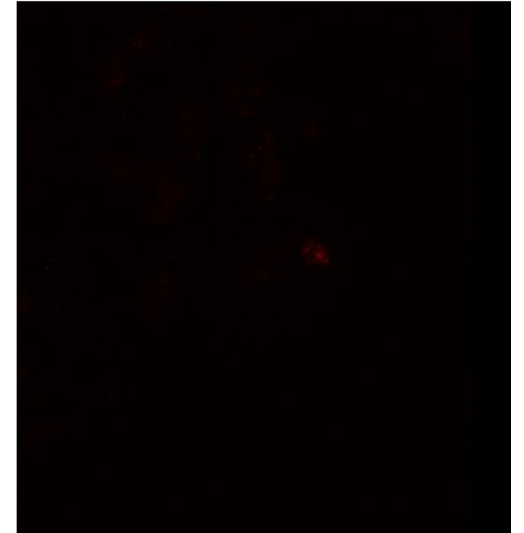
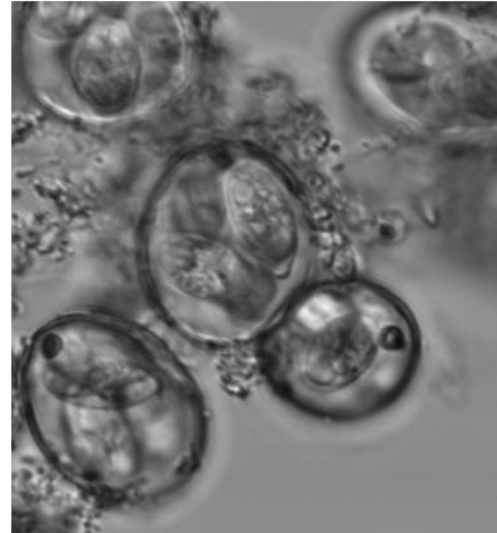
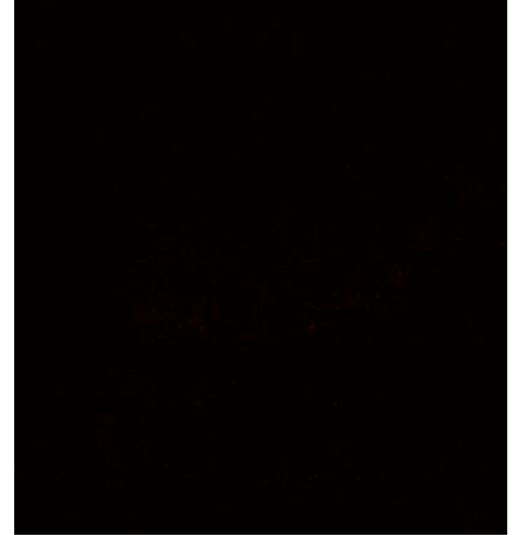
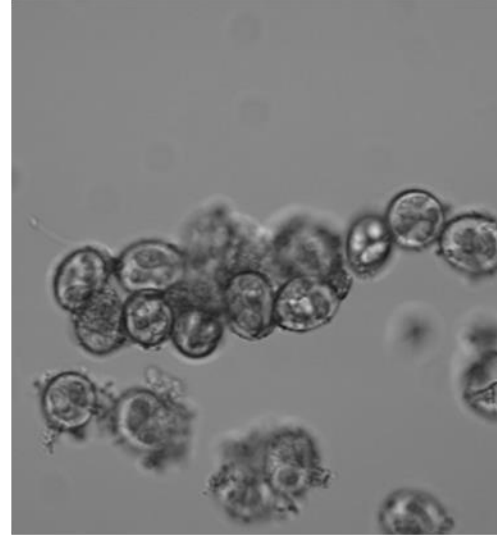
TA4-3



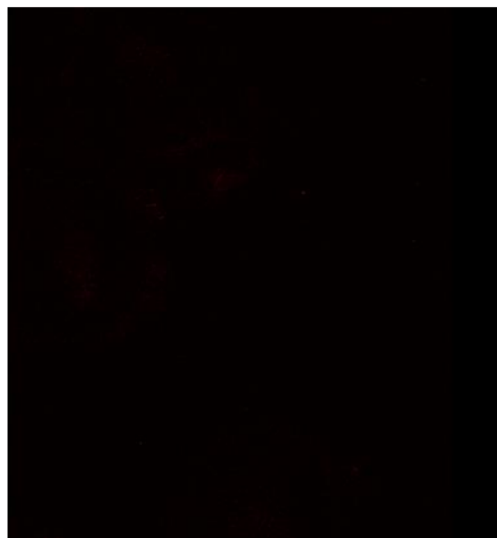
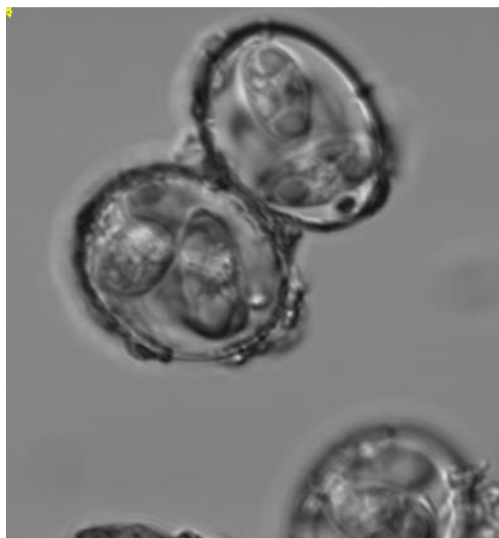
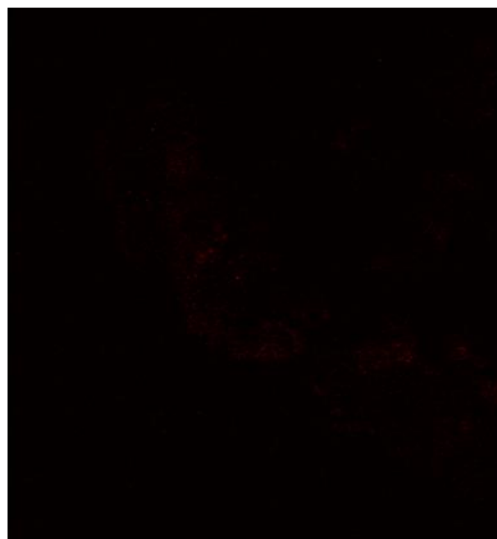
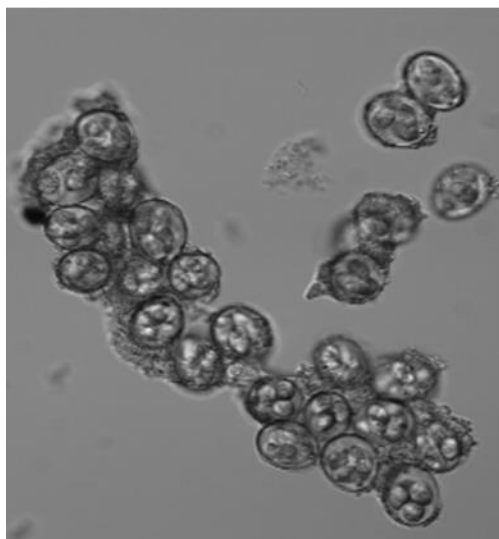
TA4-2



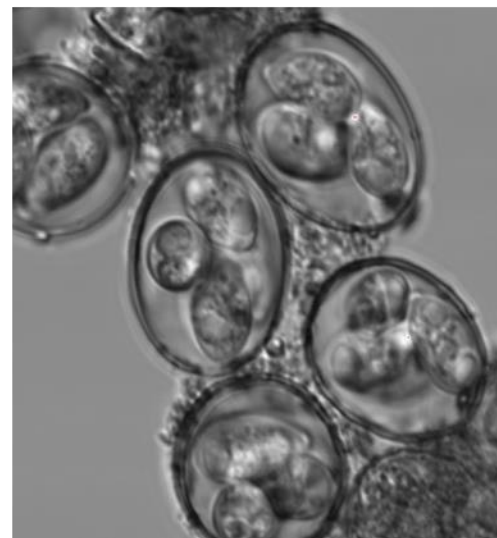
TA4-4



WC-S16

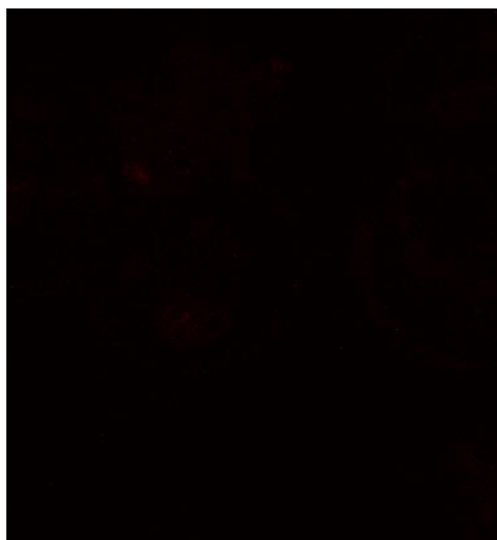
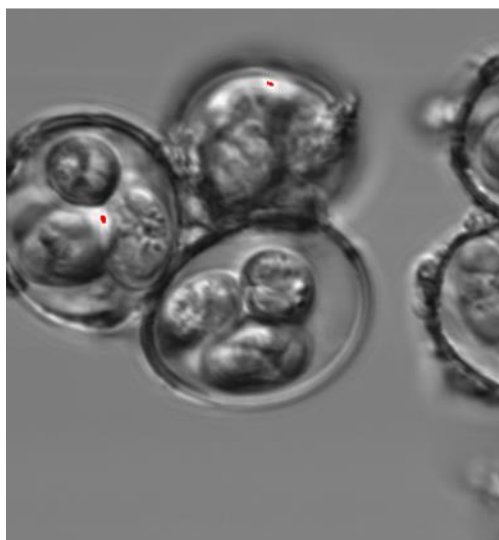
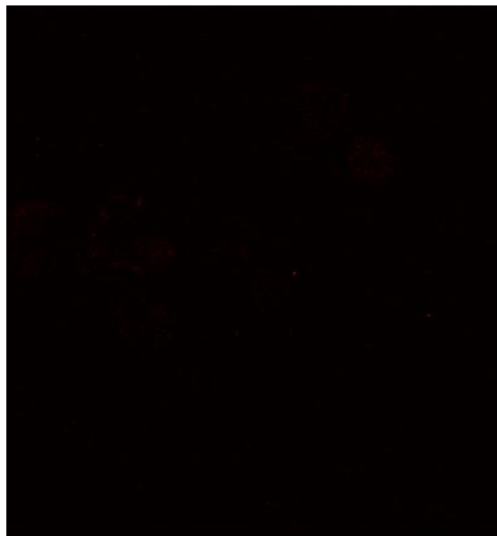
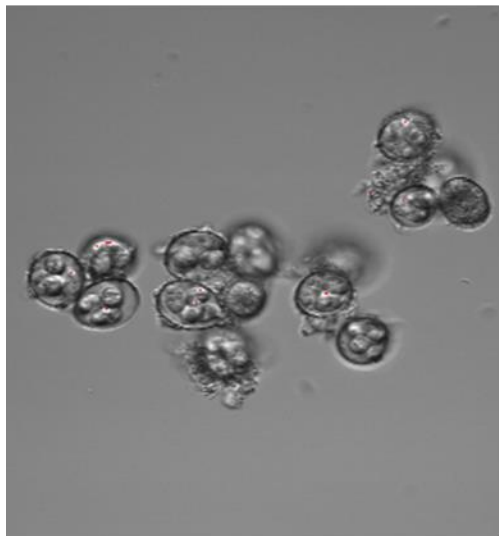


WC-S3

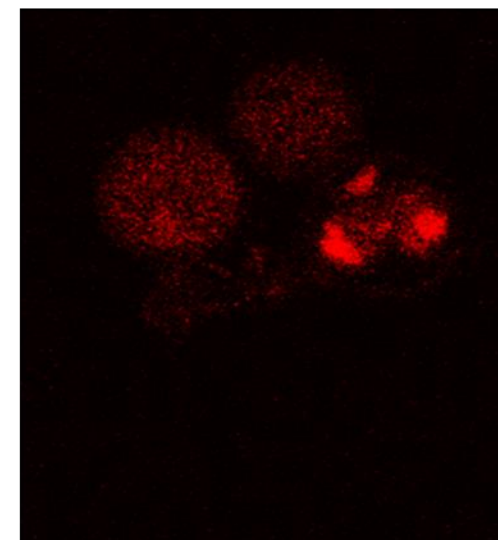
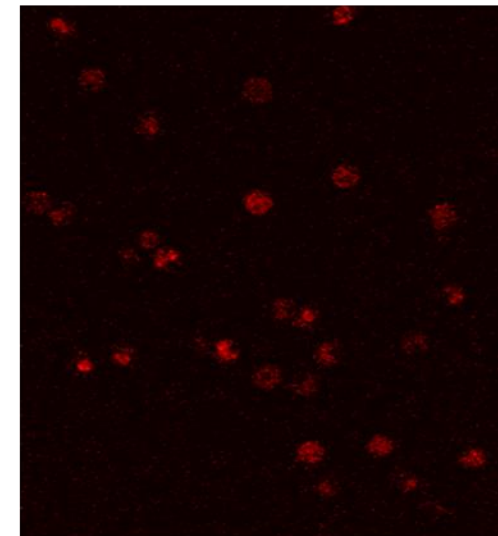
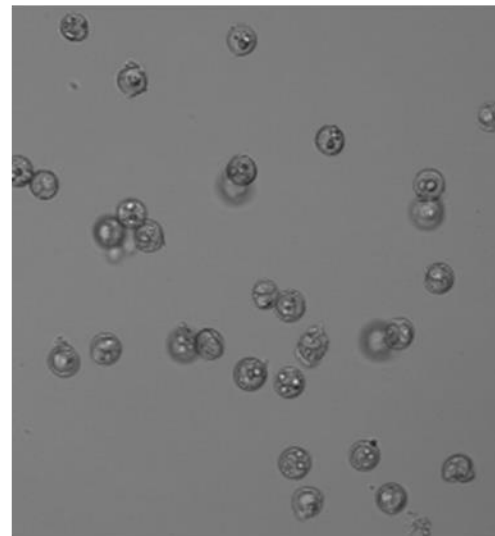


Assay Controls

**No Aptamer
(-)ve Control**



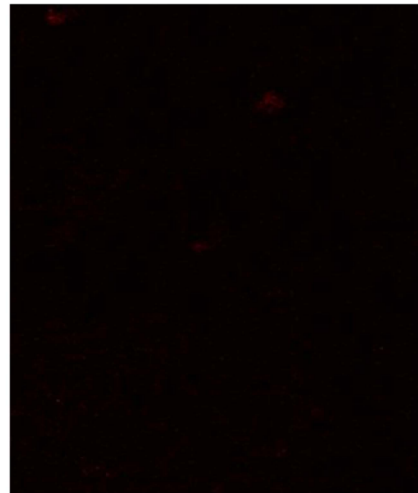
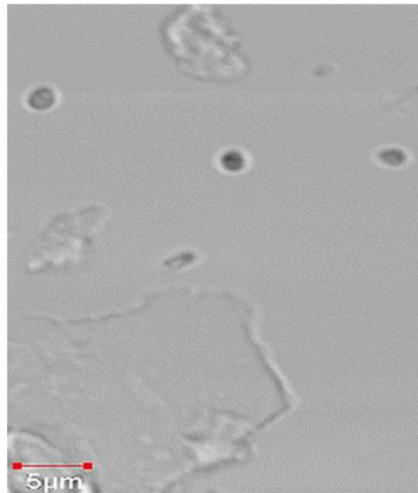
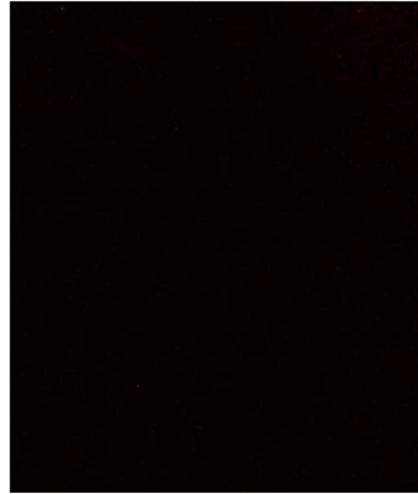
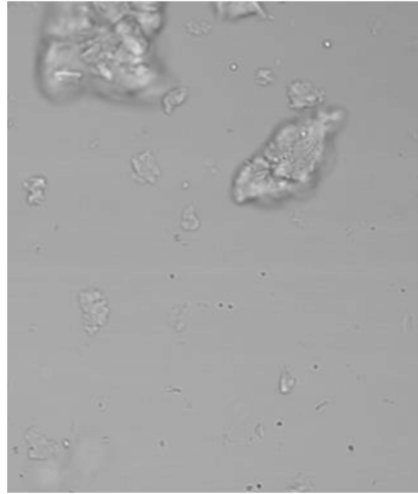
**WP2-1 + Cyclospora
(+)ve Control**



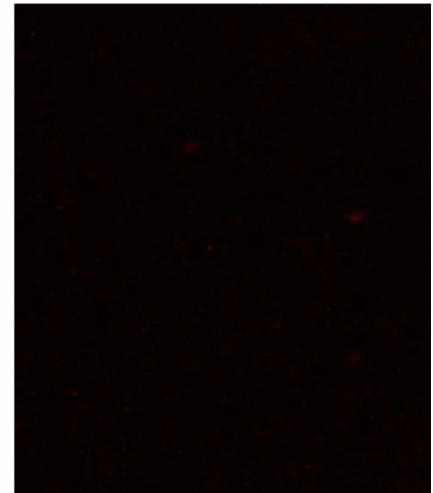
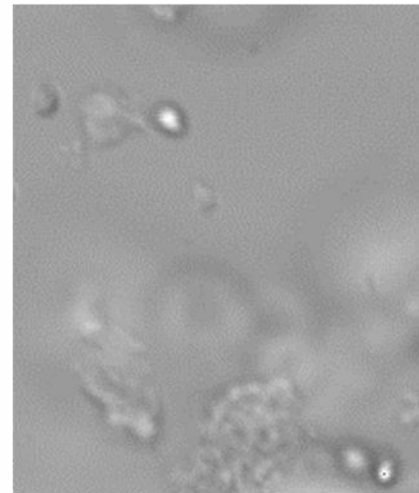
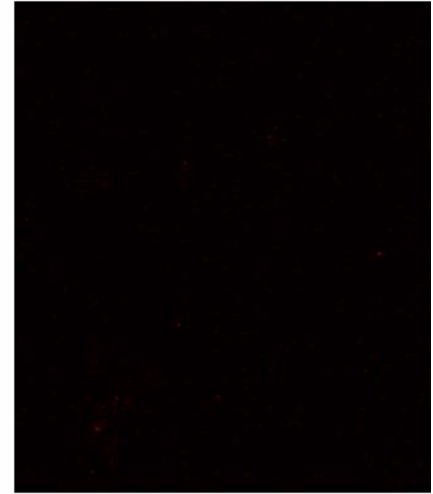
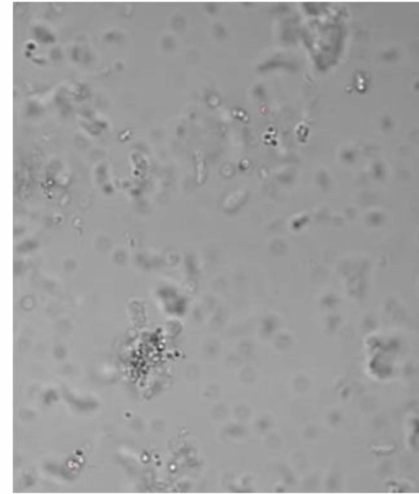
Conclusion: *Eimeria tenella* is negative with all aptamers. There is no positive signal.

Giardia Lamblia

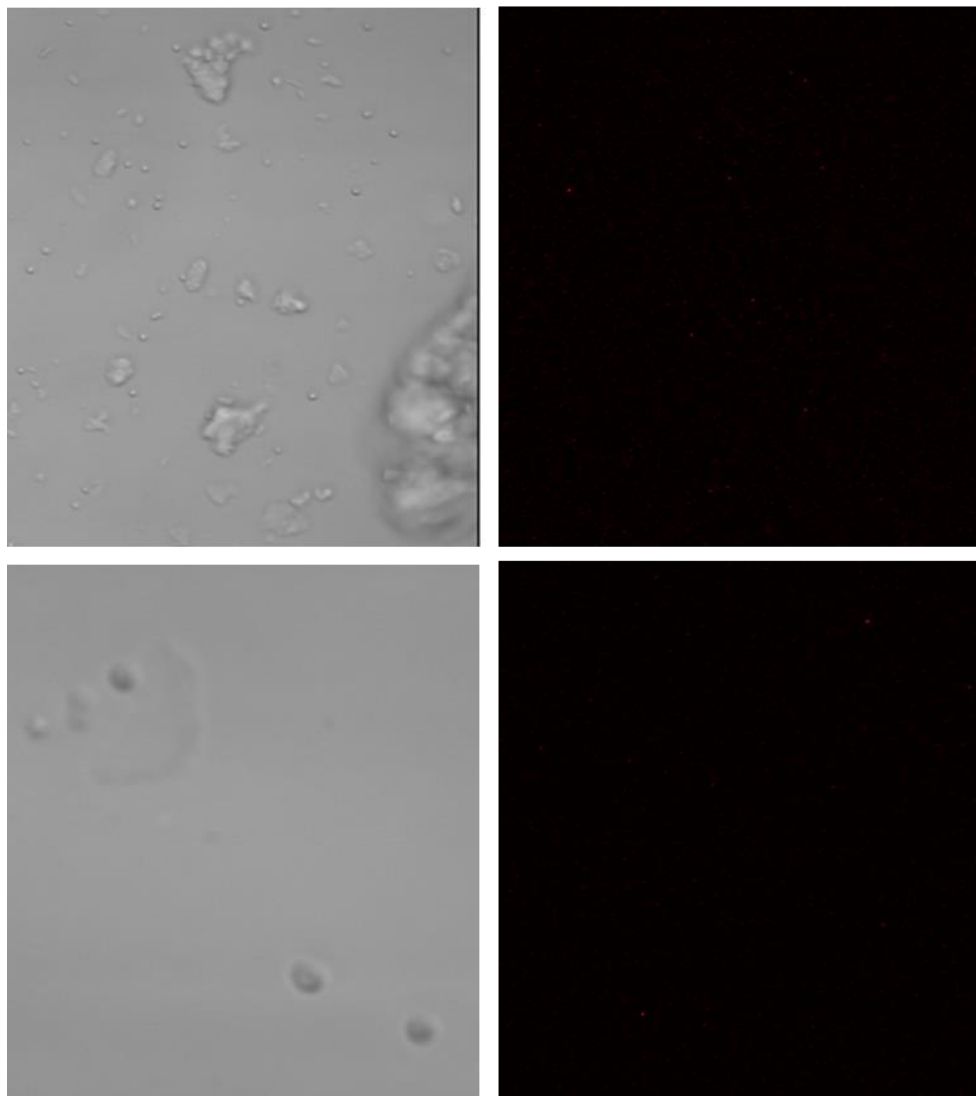
WP2-1



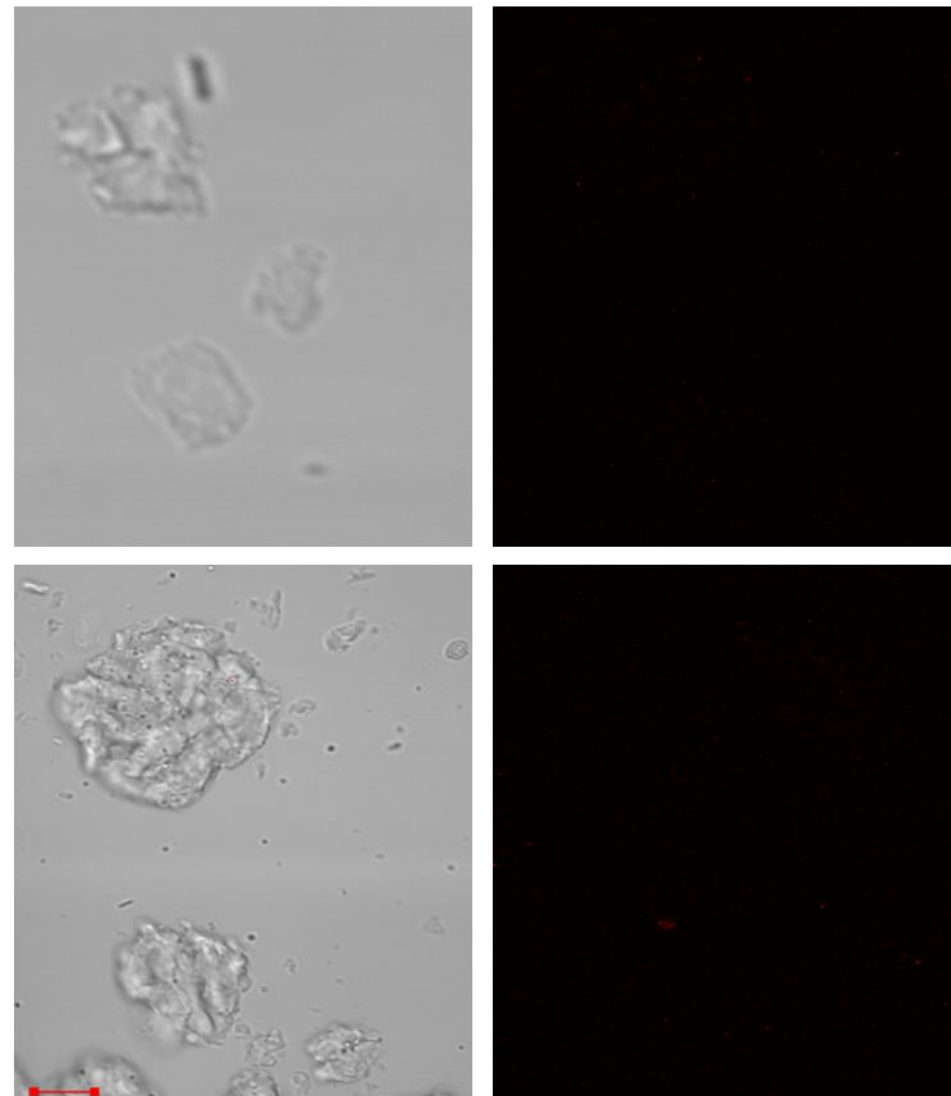
WP2-4



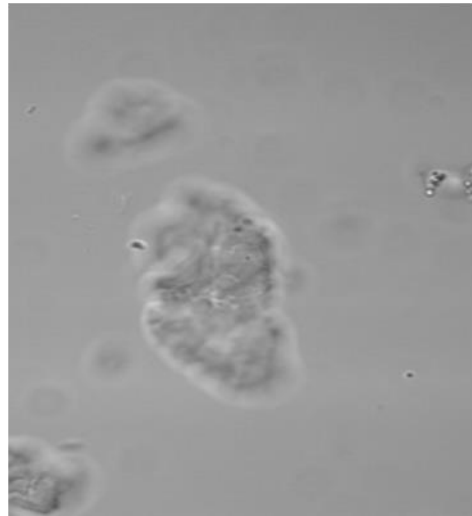
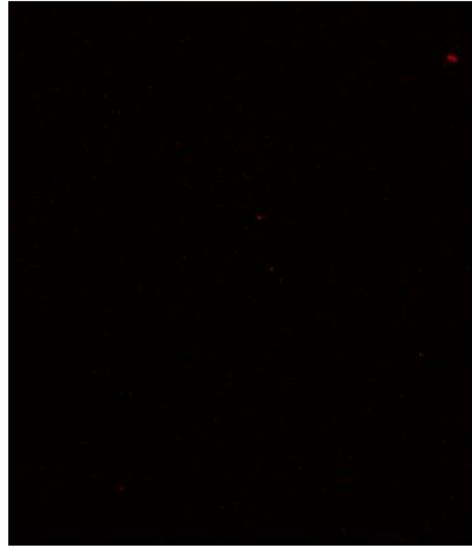
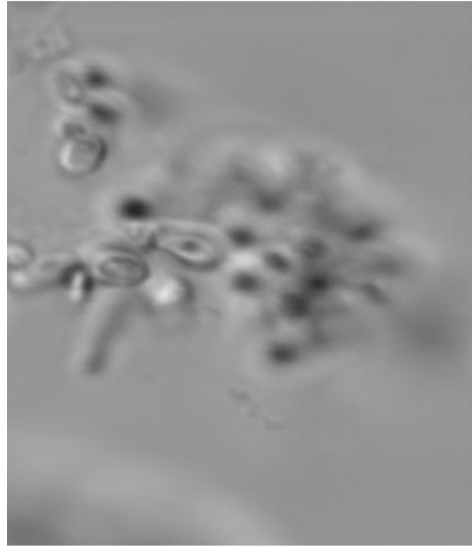
WP2-2



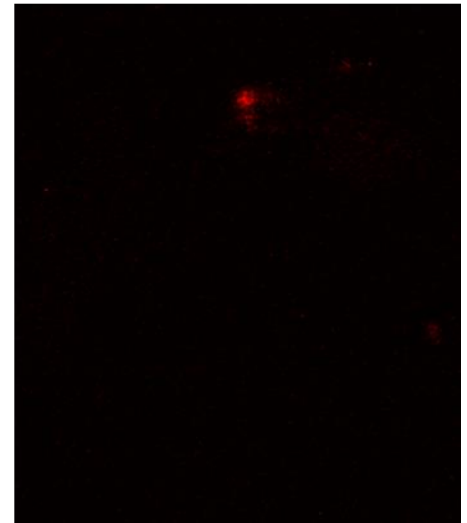
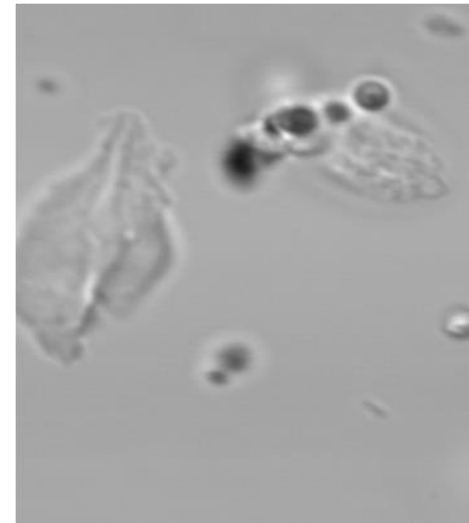
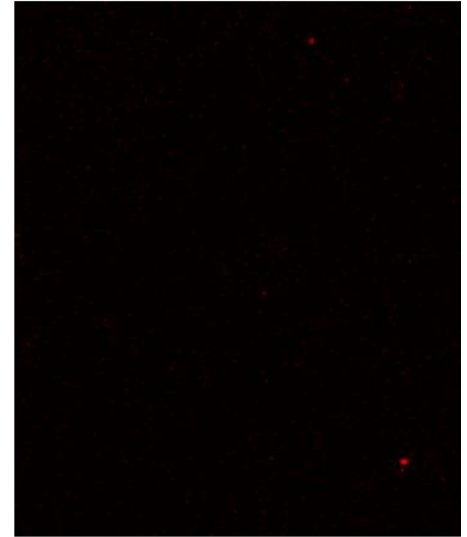
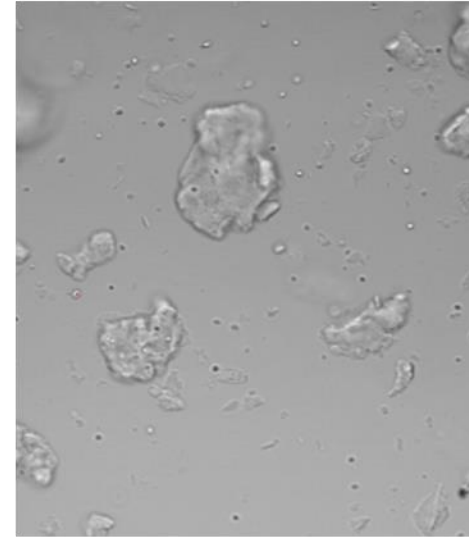
WP2-3



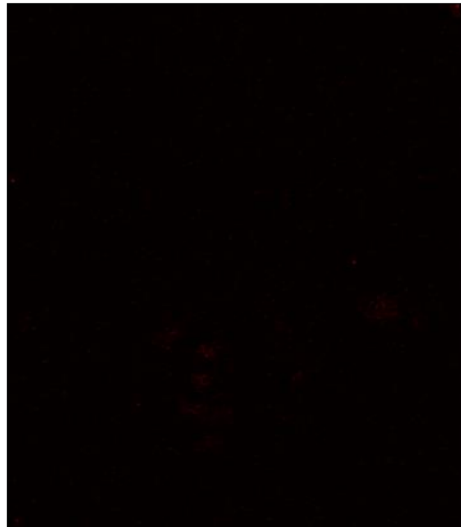
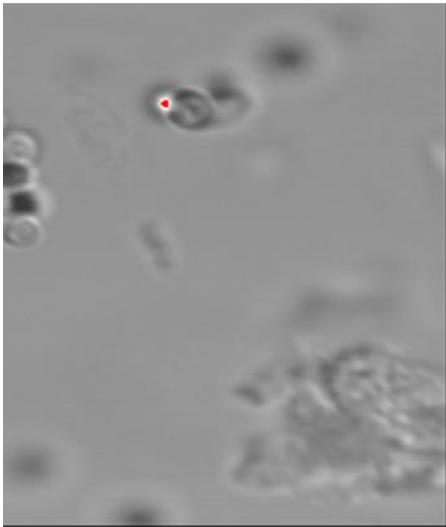
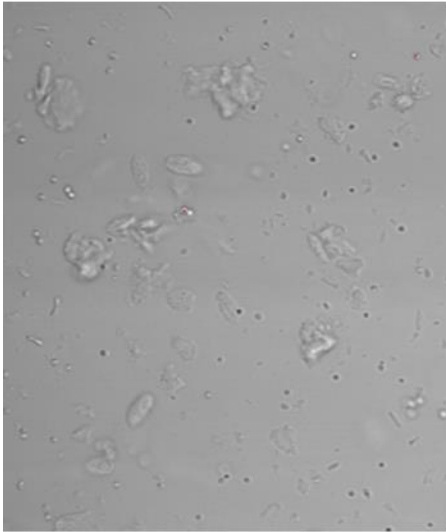
TA4-1



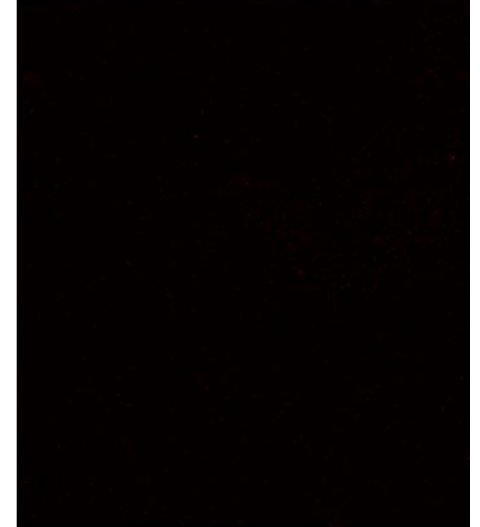
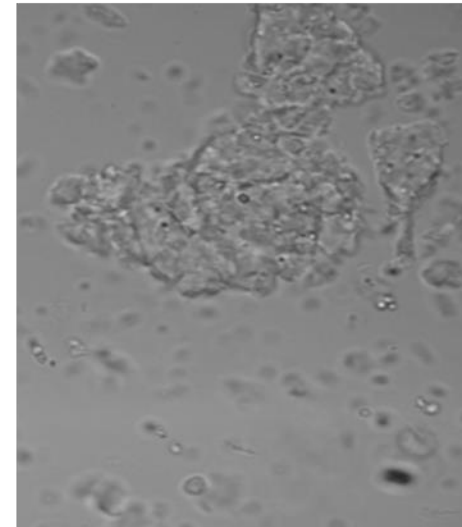
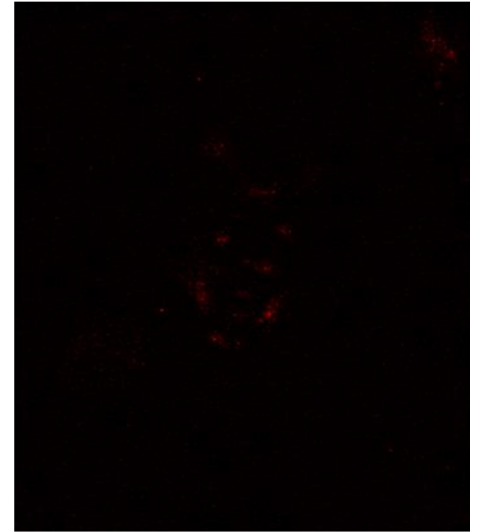
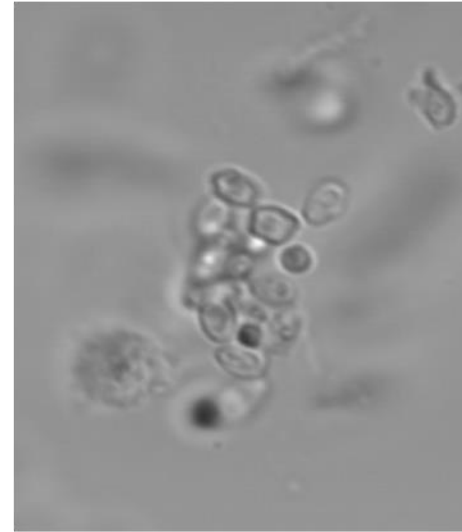
TA4-3



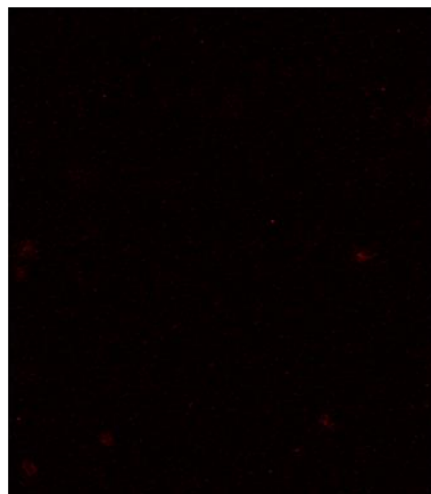
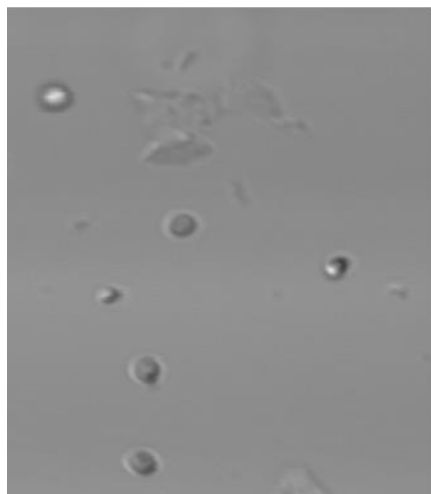
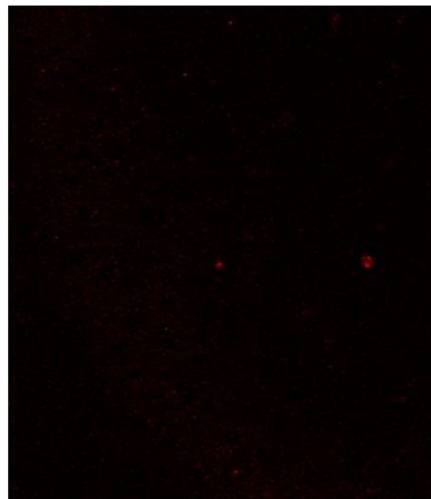
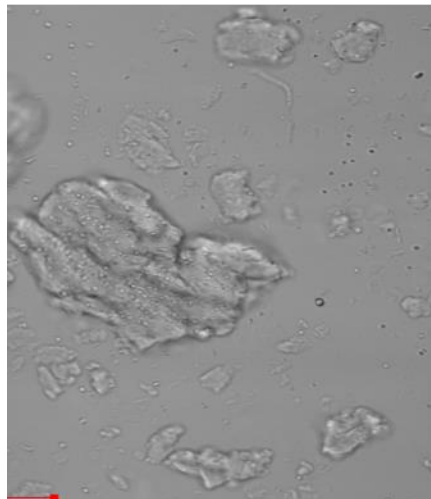
TA4-2



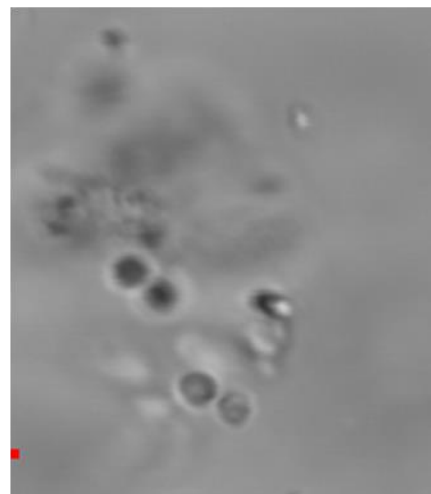
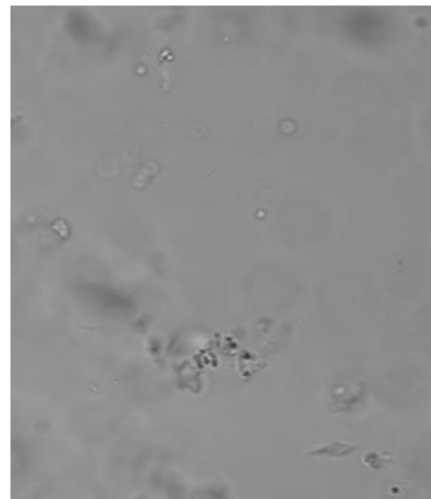
TA4-4



Whole Cyclo S16

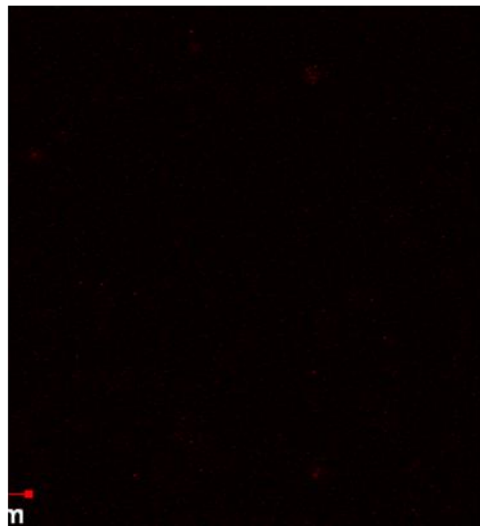
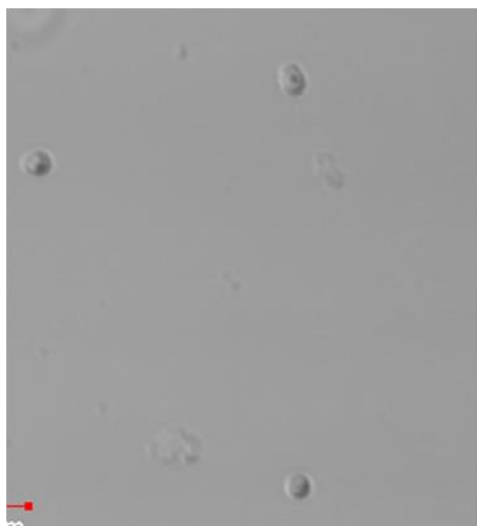
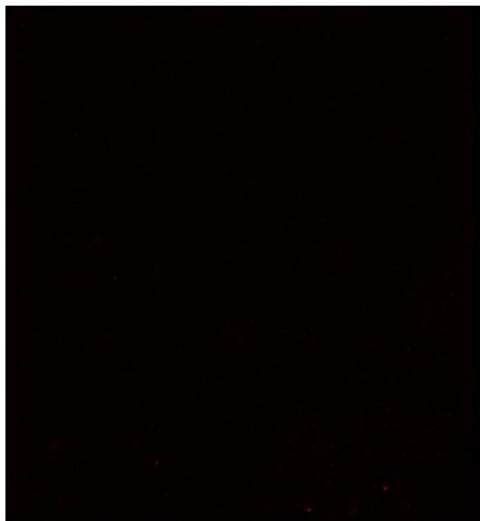
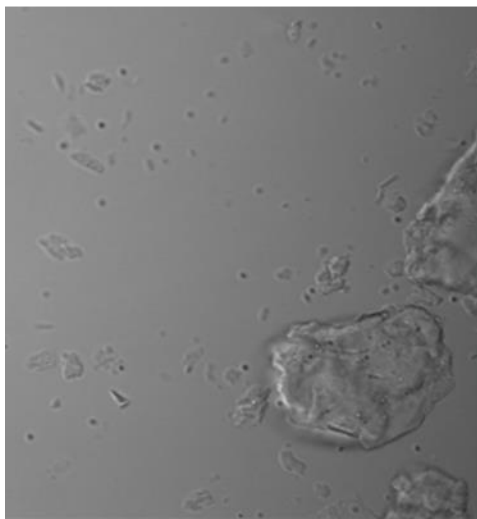


Whole Cyclo S3

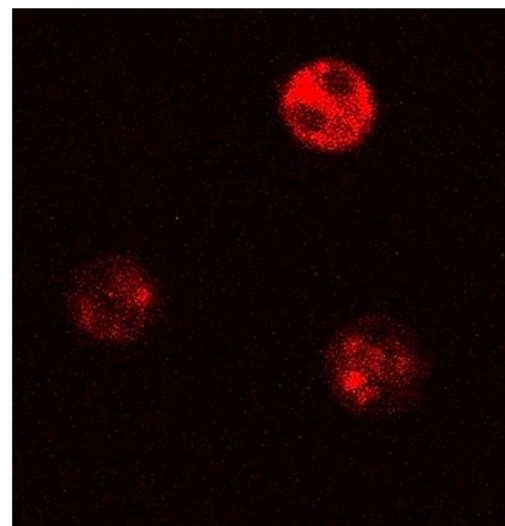
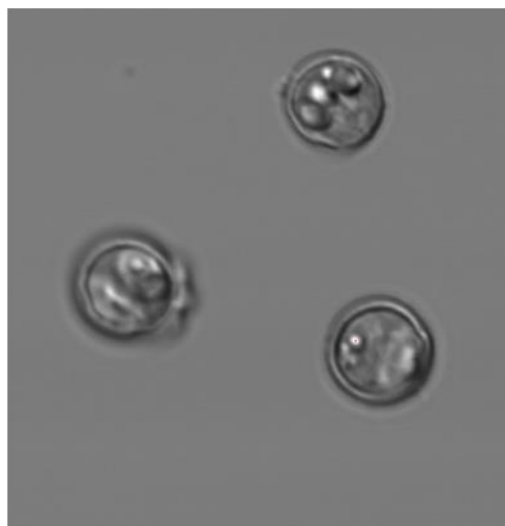
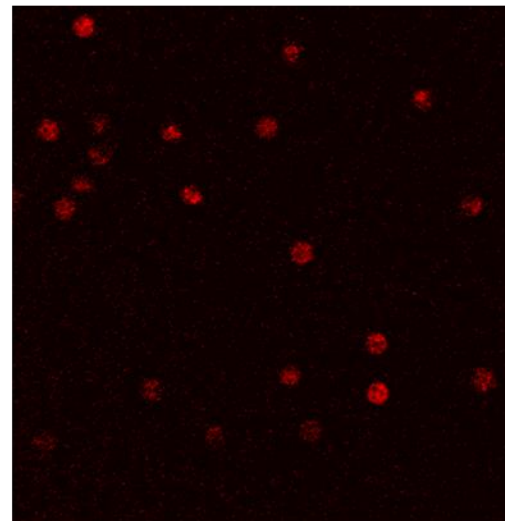
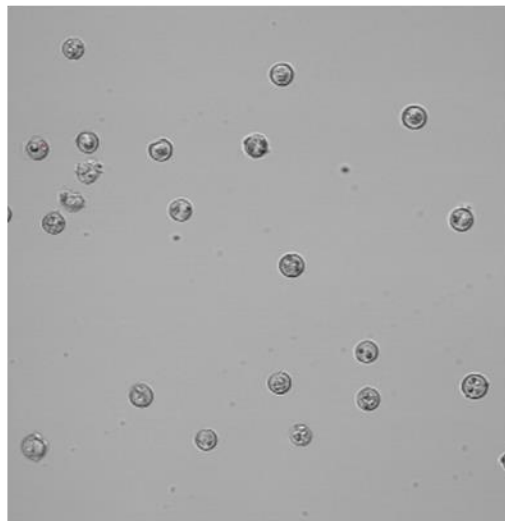


Assay Controls

**No Aptamer
(-)ve Control**



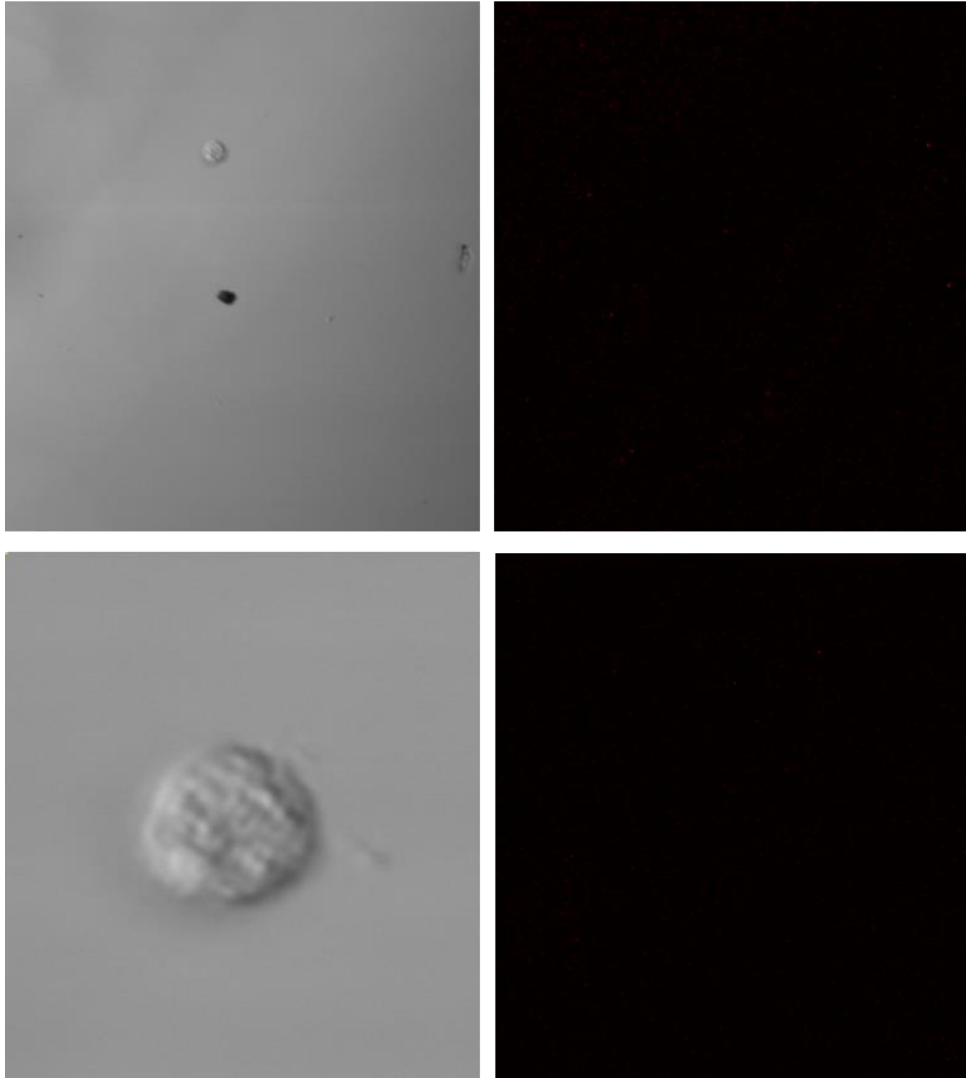
**WP2-1 + Cyclospora
(+)ve Control**



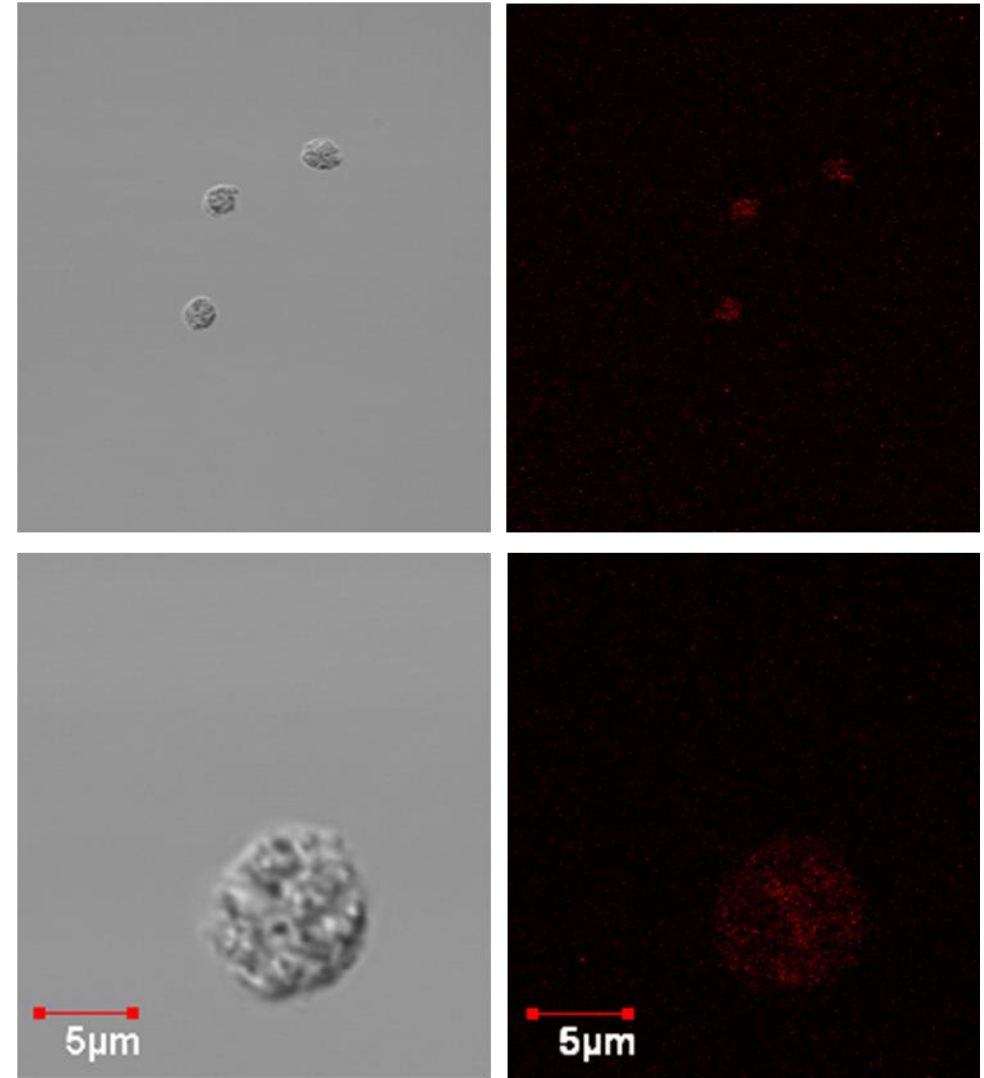
Conclusion: Giardia lamblia showed no positive signals with any of the aptamers.

Naeglaria Fowleri

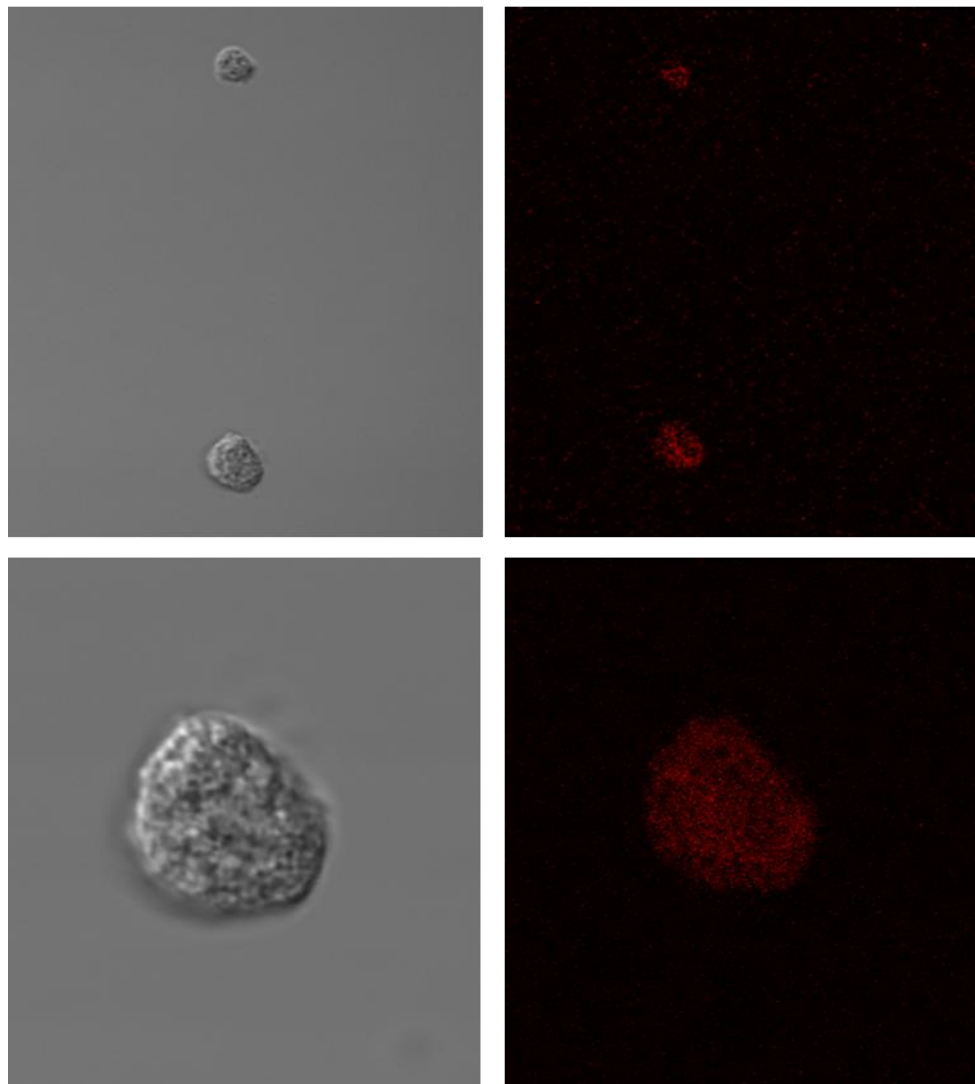
WP2-1



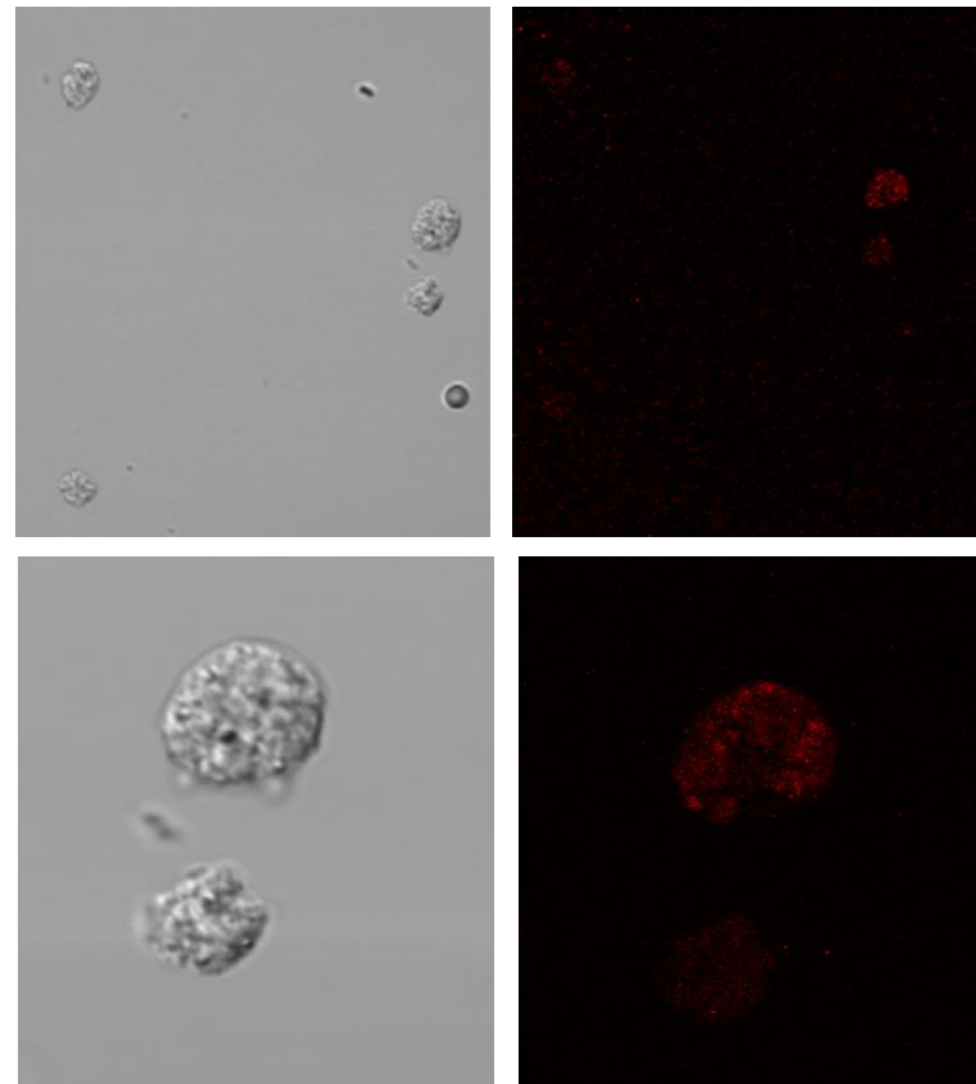
WP2-4



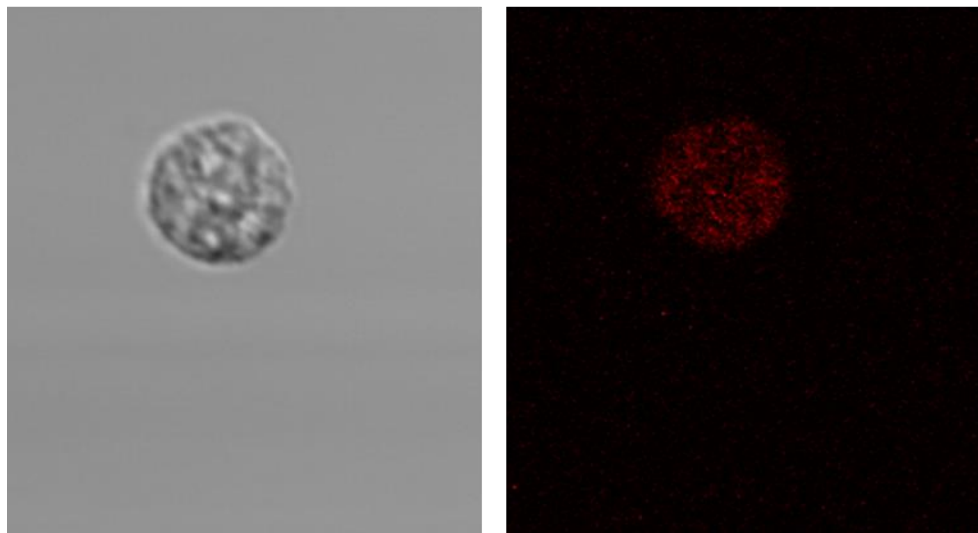
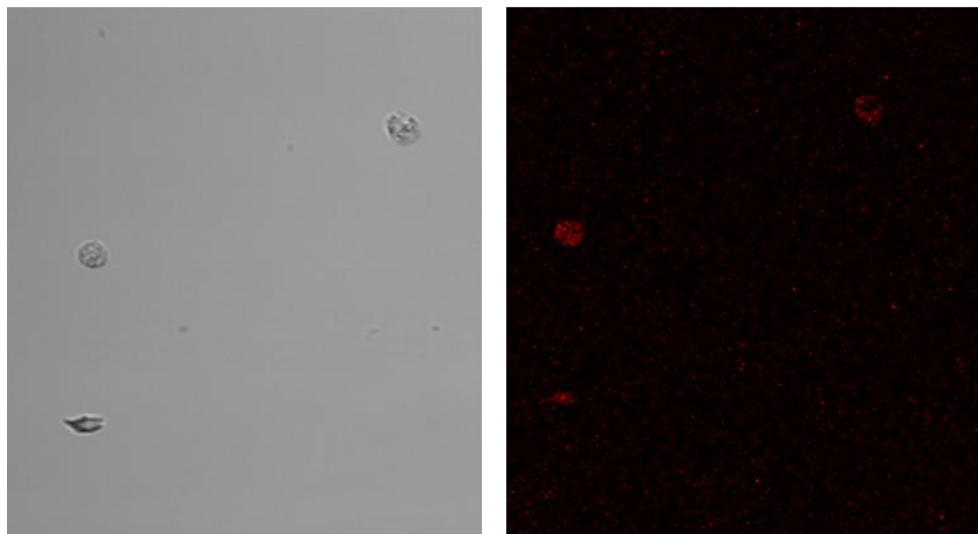
WP2-2



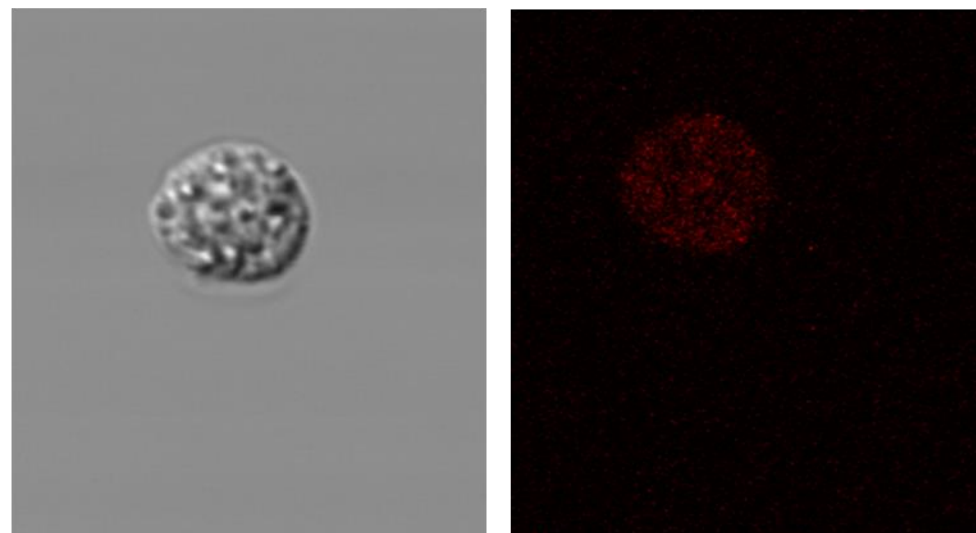
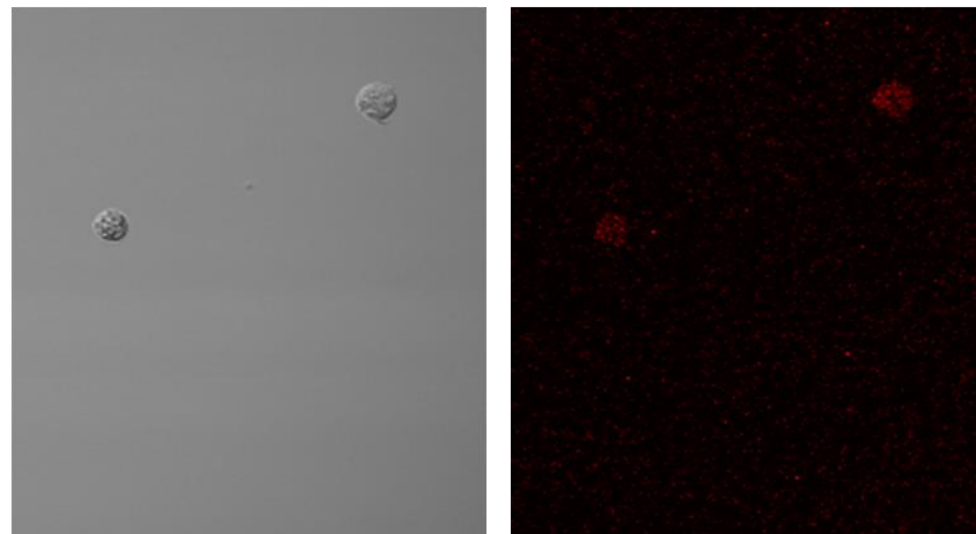
WP2-3



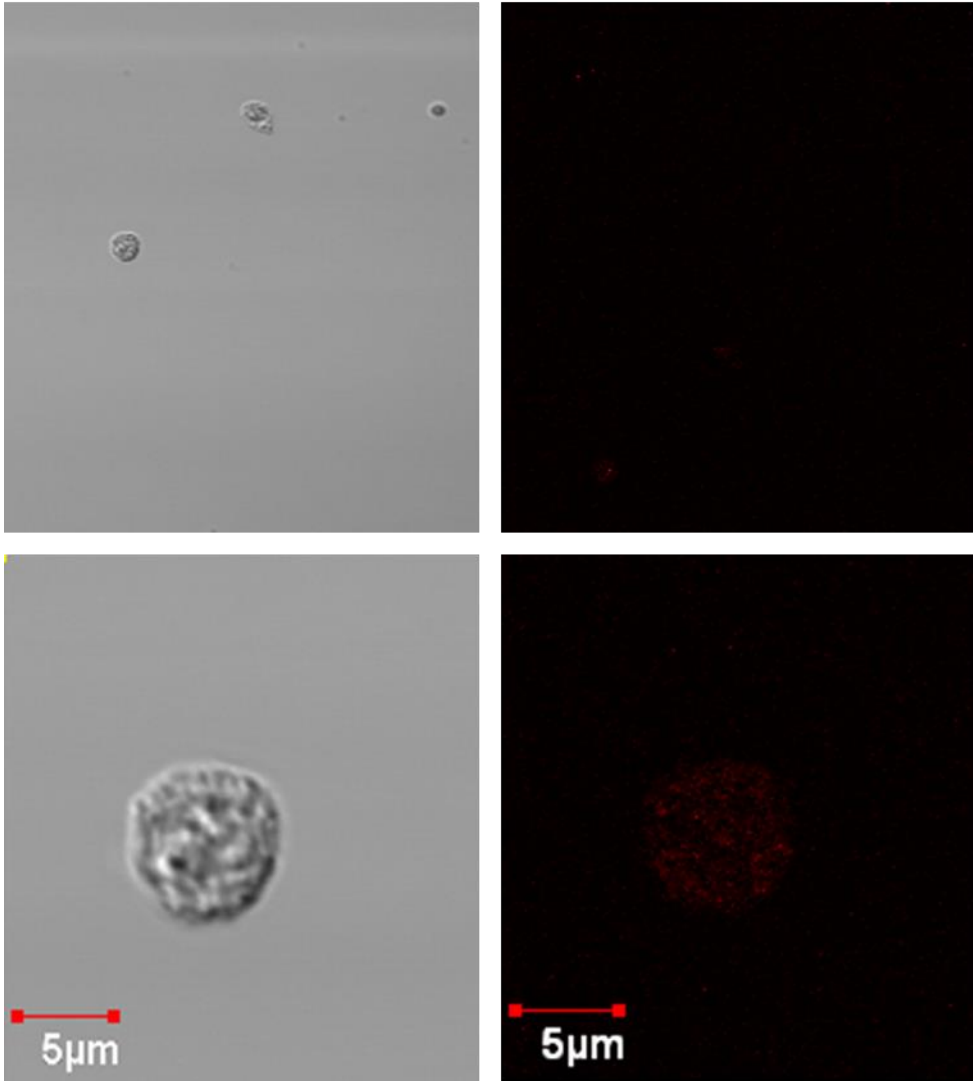
TA4-1



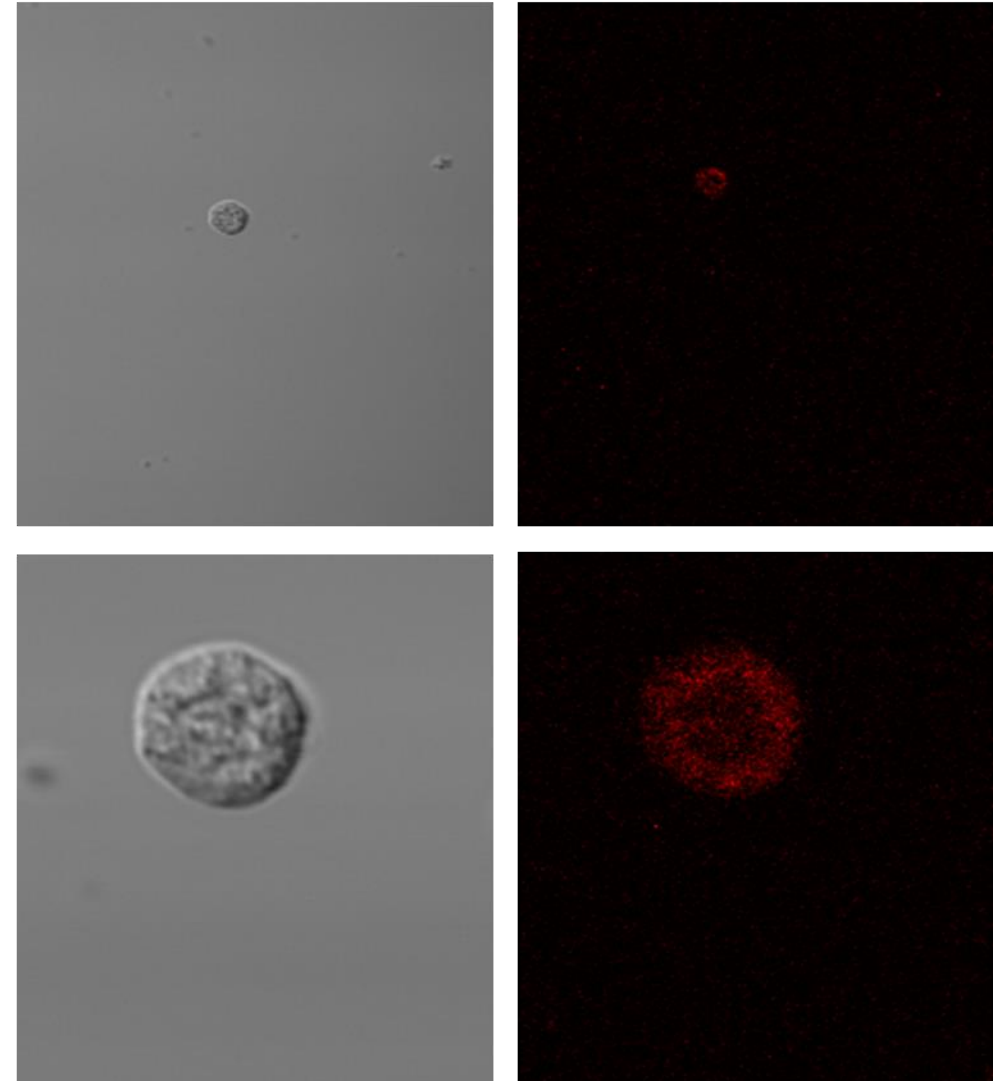
TA4-3



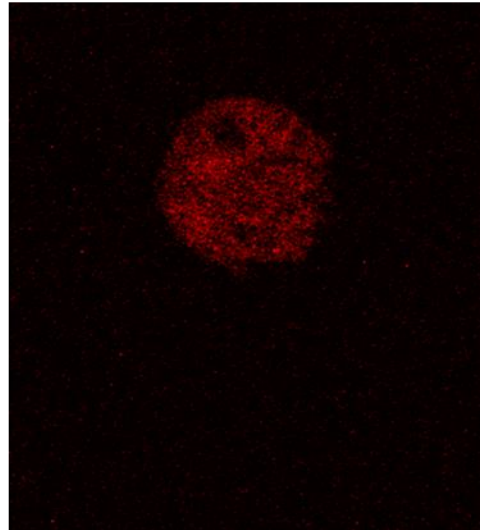
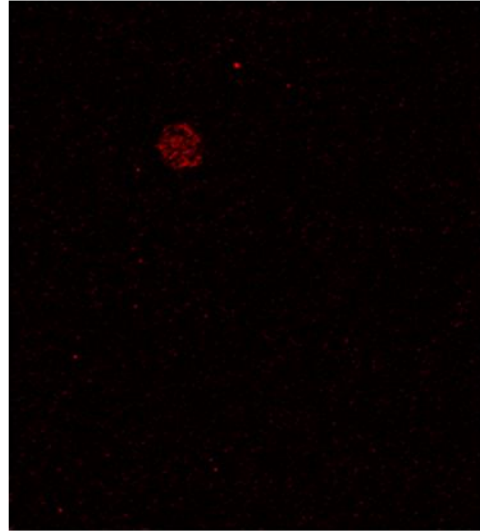
TA4-2



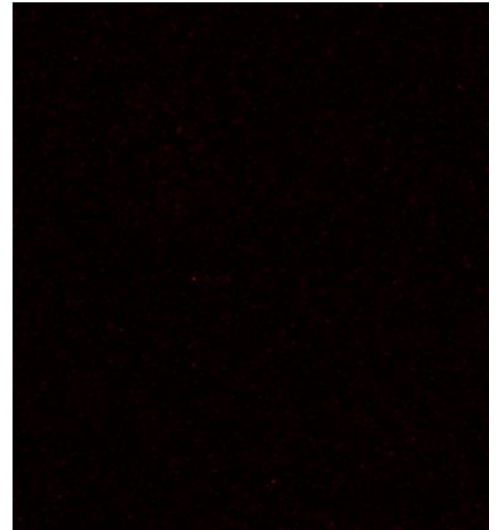
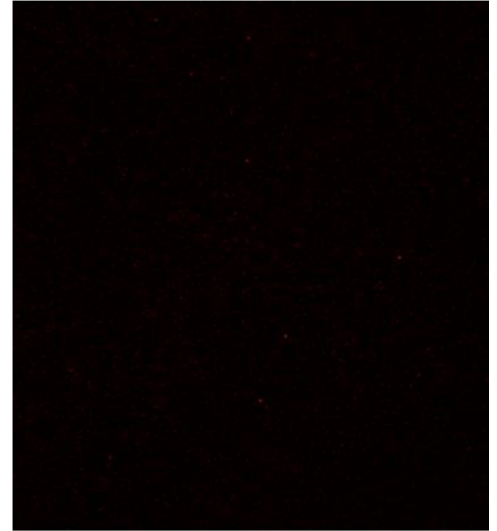
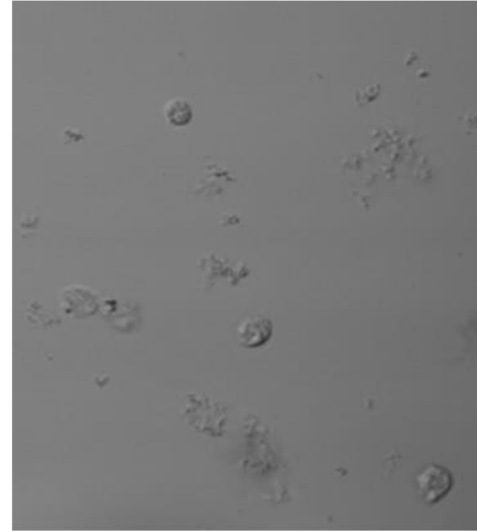
TA4-2



WC-S16

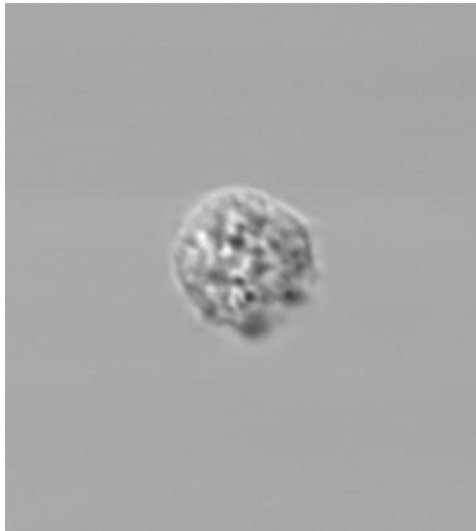


WC-S3

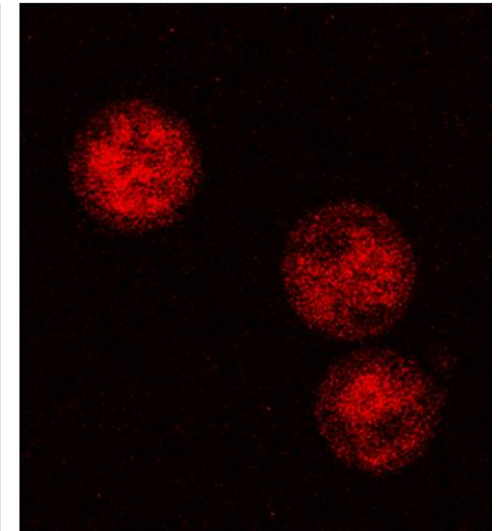
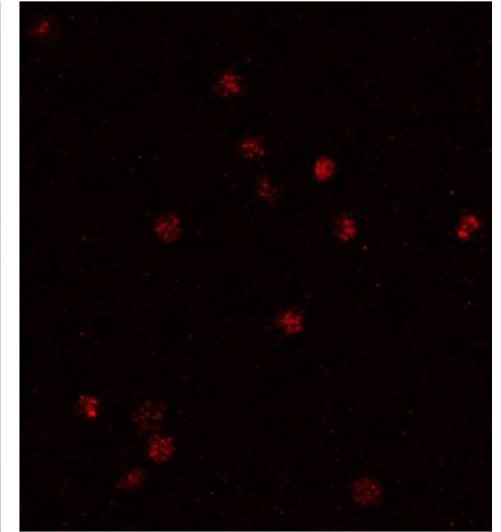


Assay Controls

**No Aptamer
(-)ve Control**



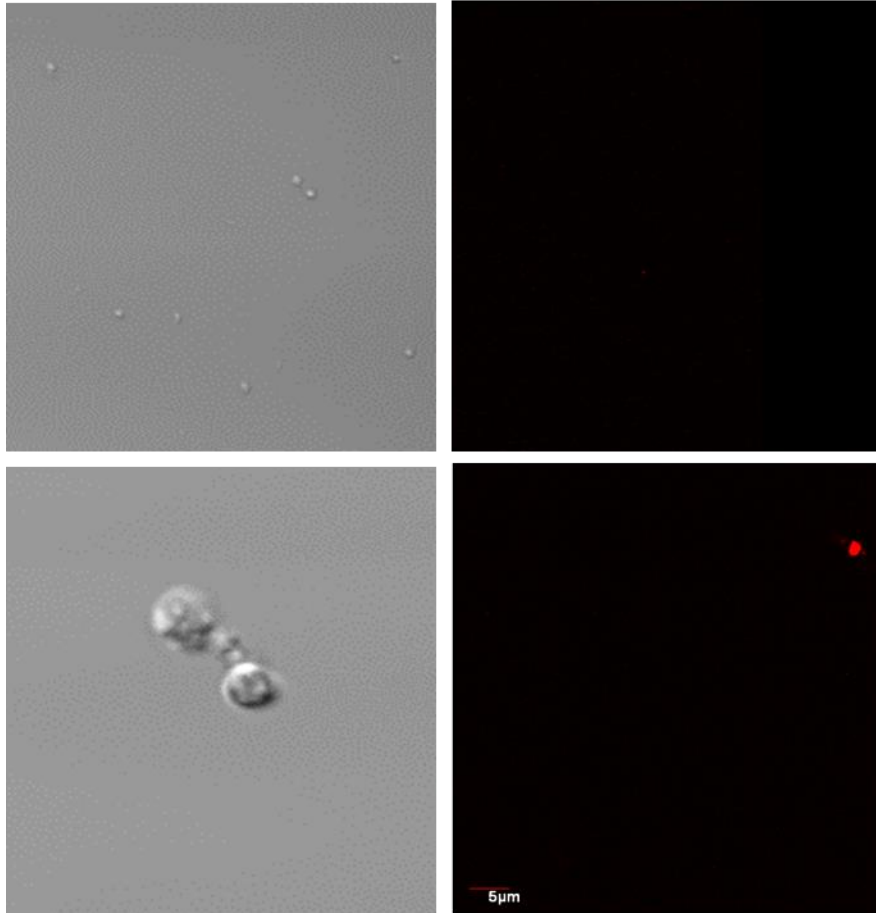
**WP2-1 + Cyclospora
(+)ve Control**



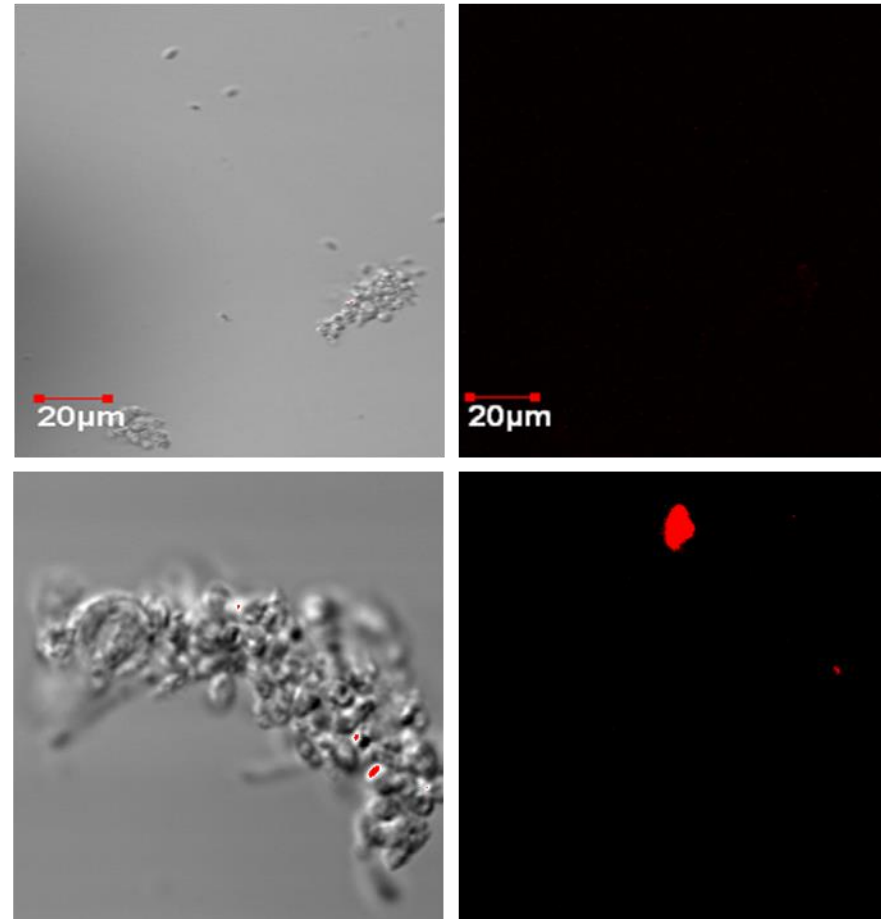
Conclusion: Except for WP2-2 and WC-S3, all aptamer showed a slight positive signals for *Naeglaria fowleri*, but signals were not nearly as strong as with *Cyclospora*.

Toxoplasma Gondii

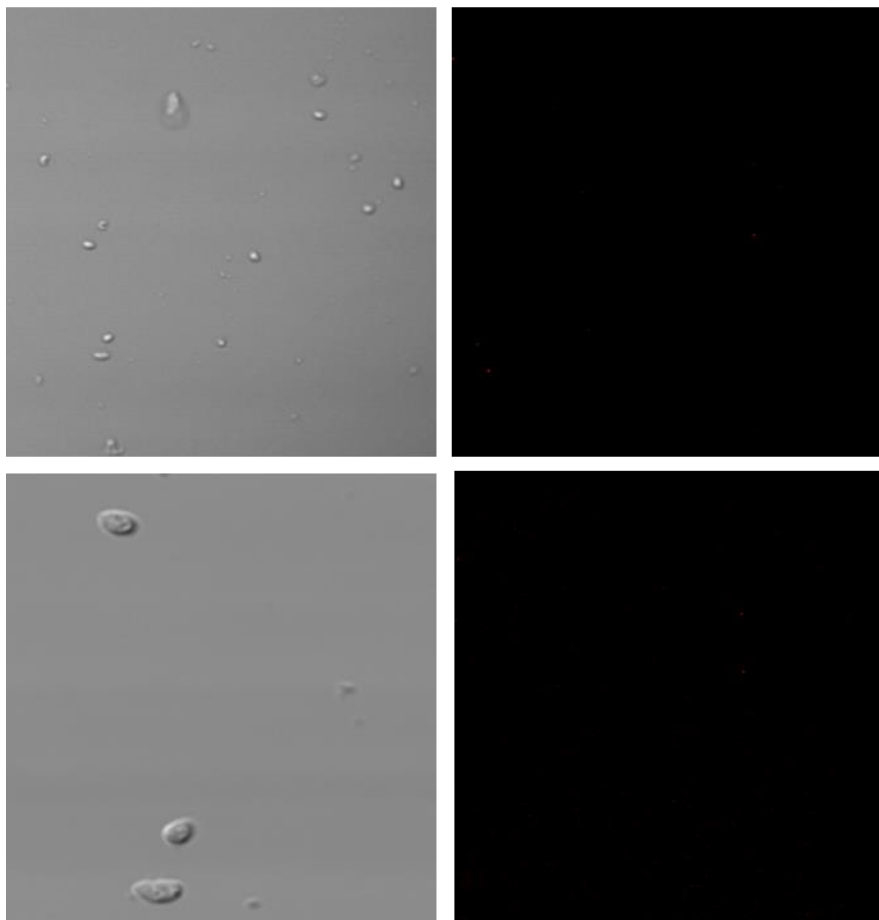
WP2-1



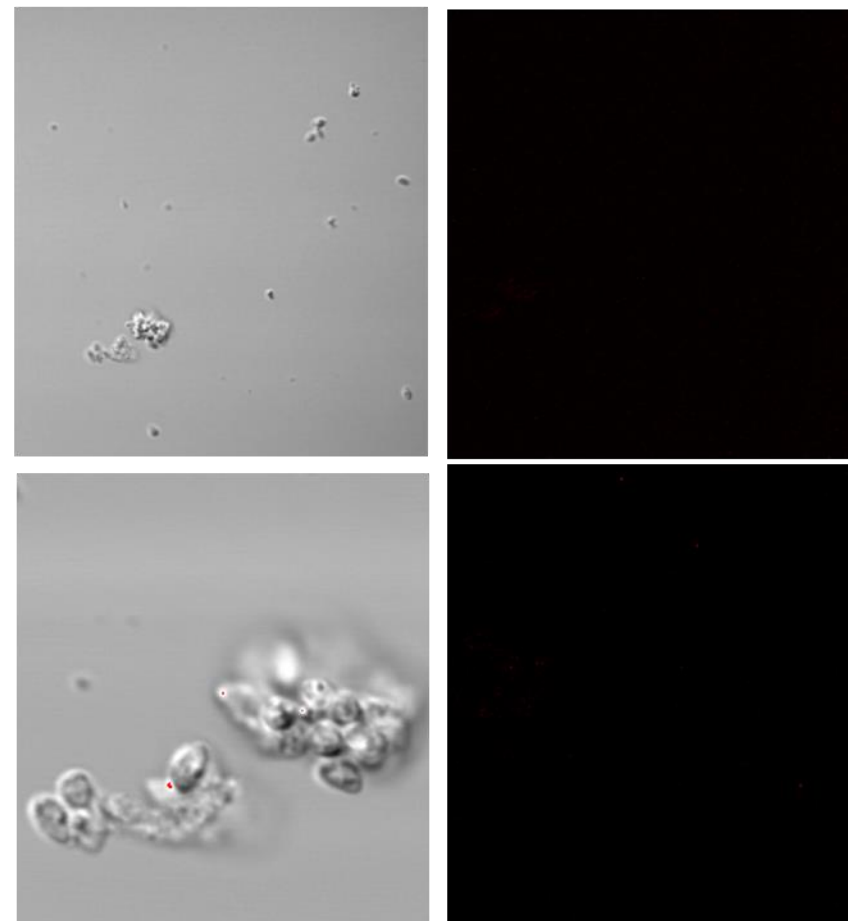
2WP2-4



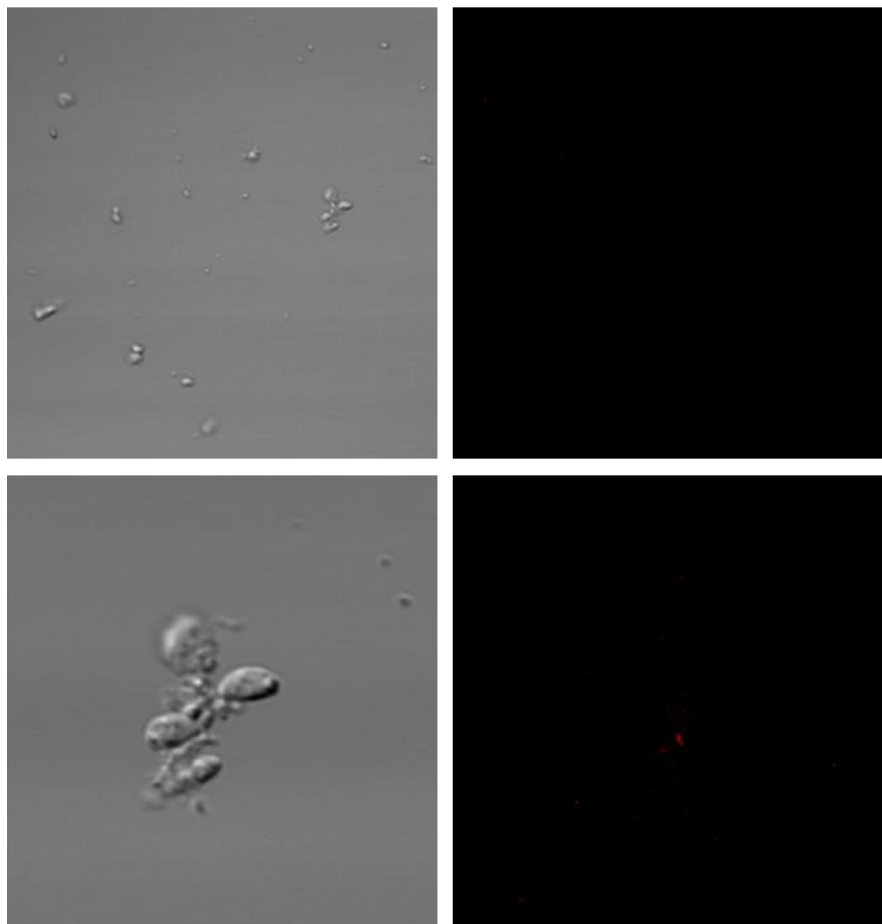
WP2-2



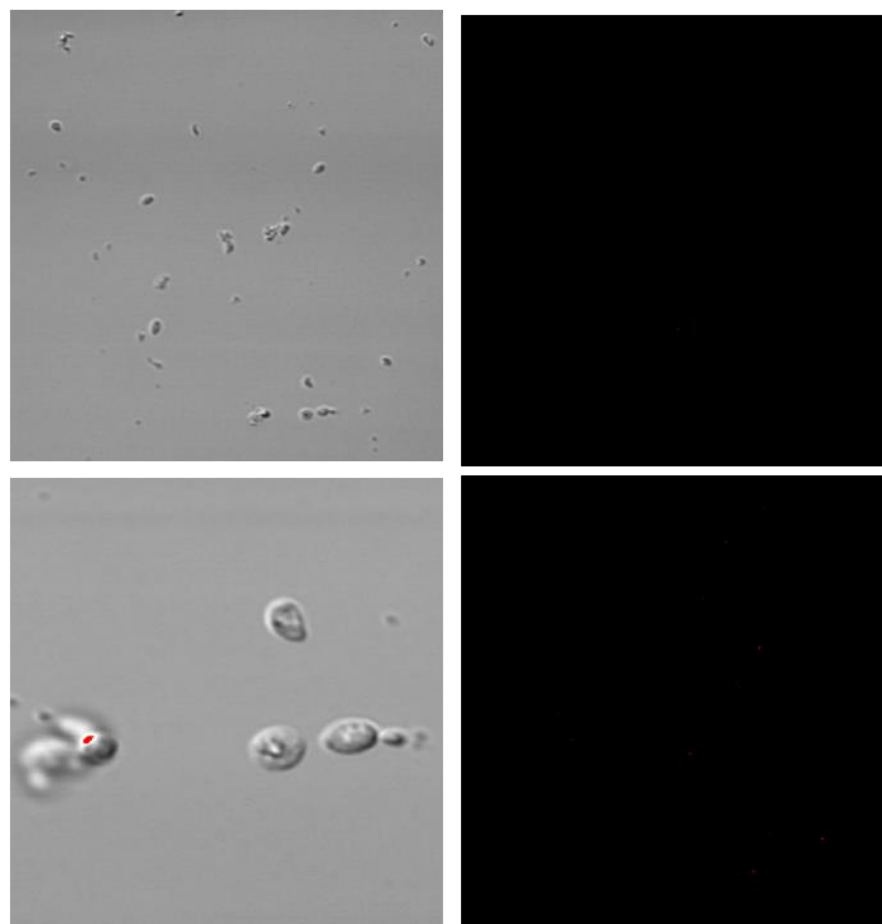
WP2-3



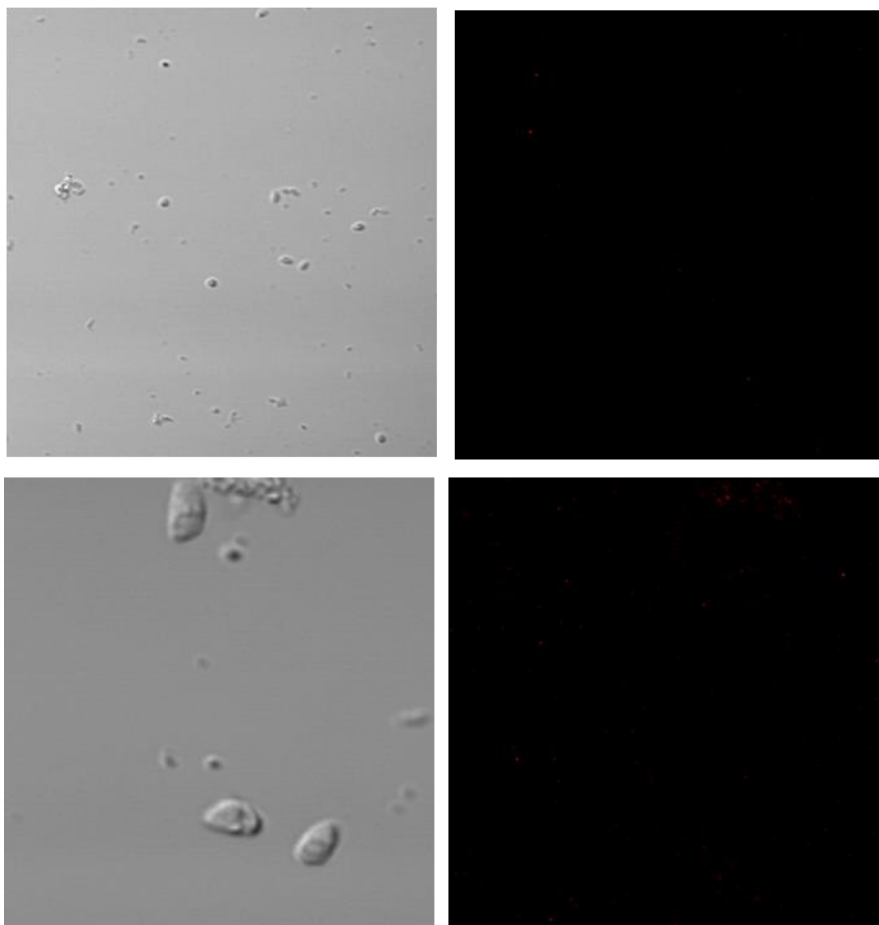
TA4-1



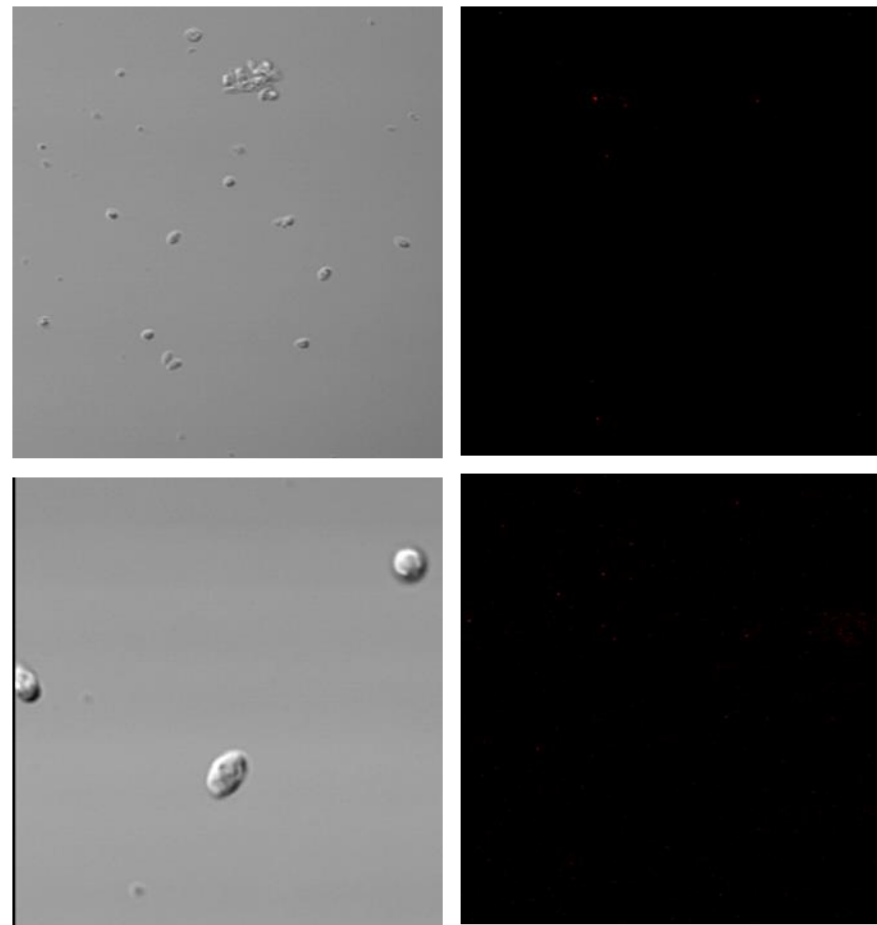
TA4-3



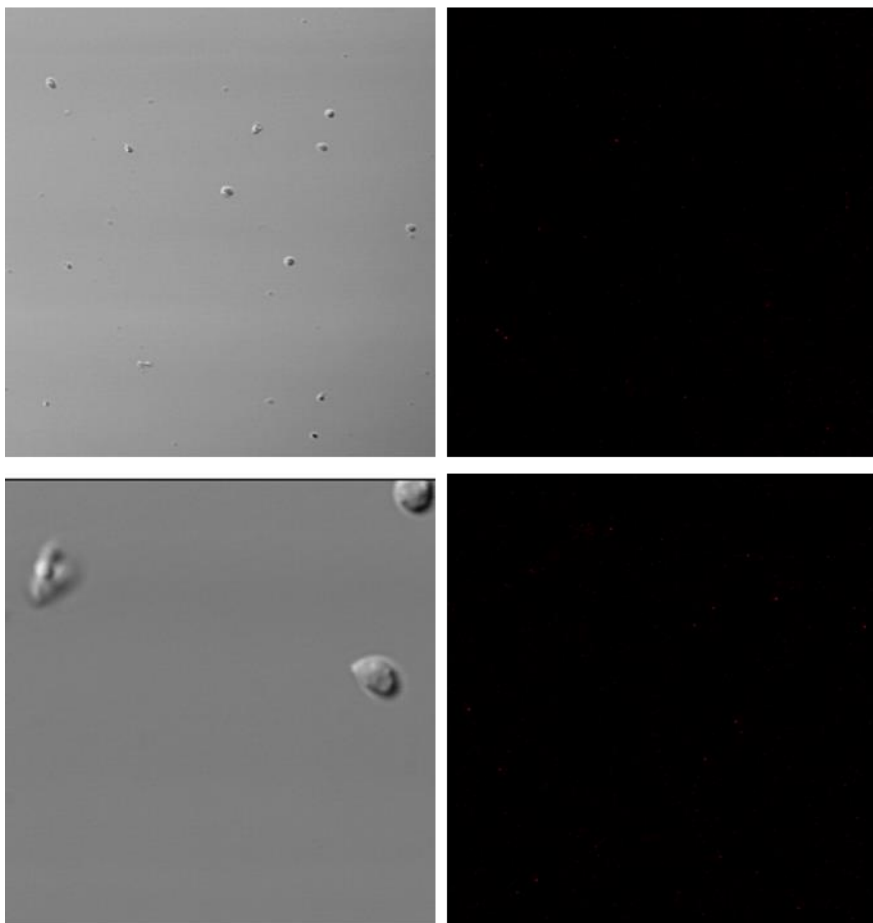
TA4-2



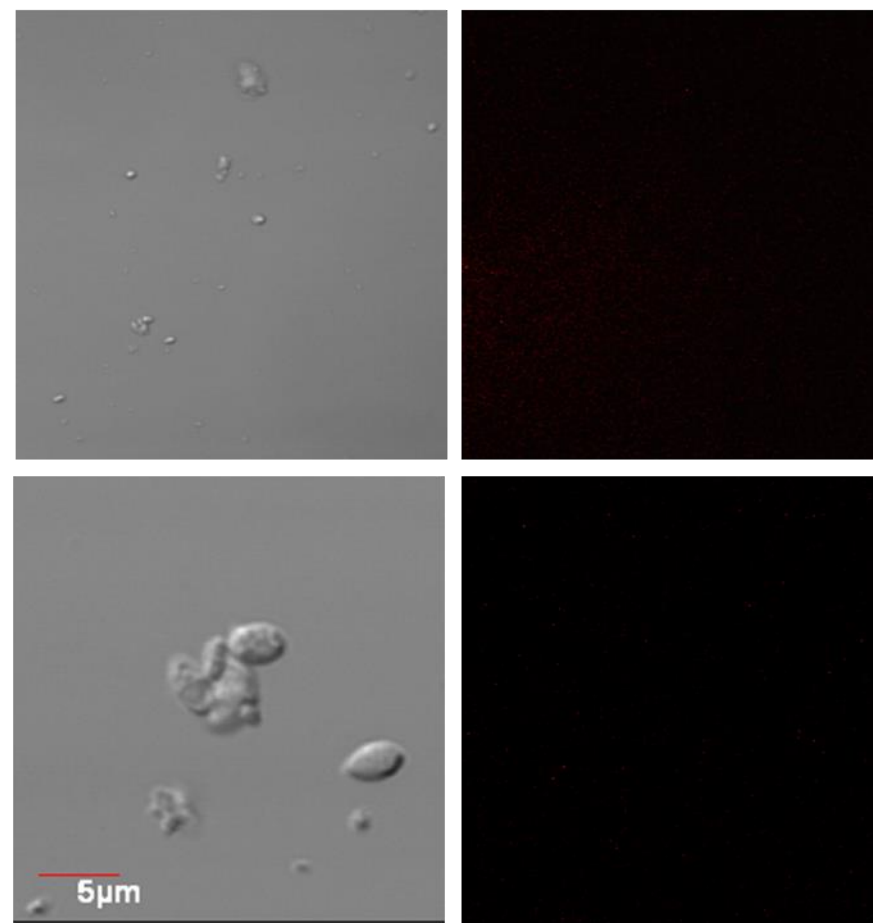
TA4-4



Whole Cyclo S16

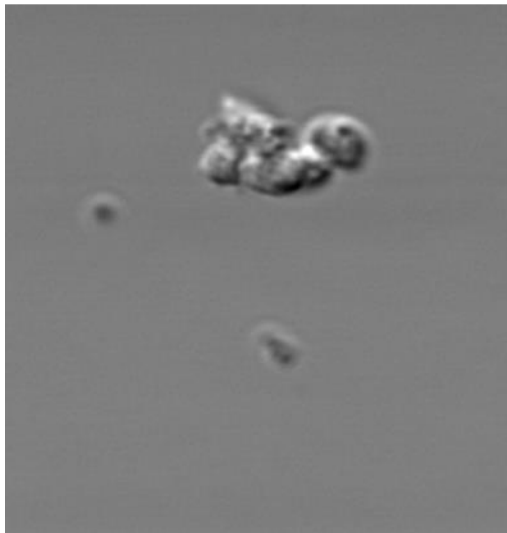
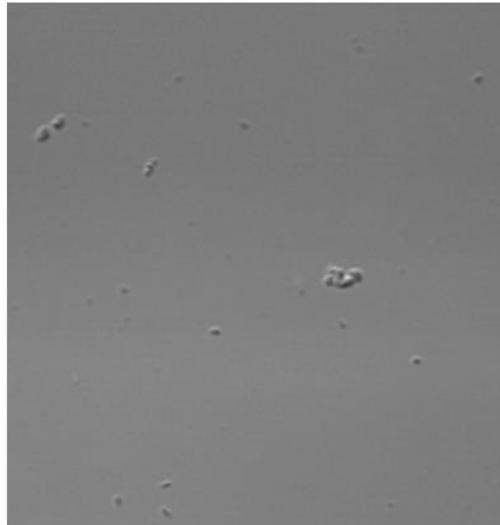


Whole Cyclo S3

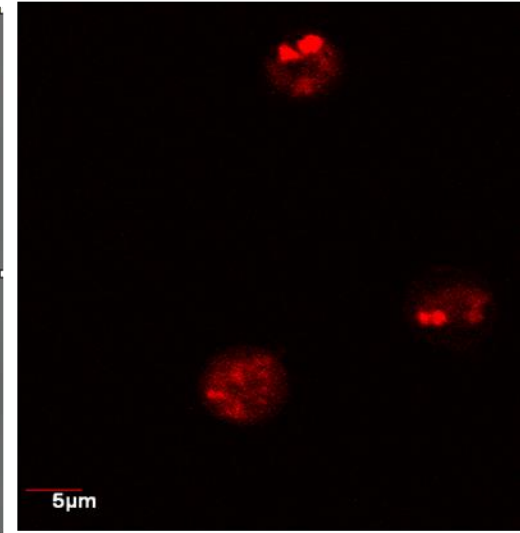
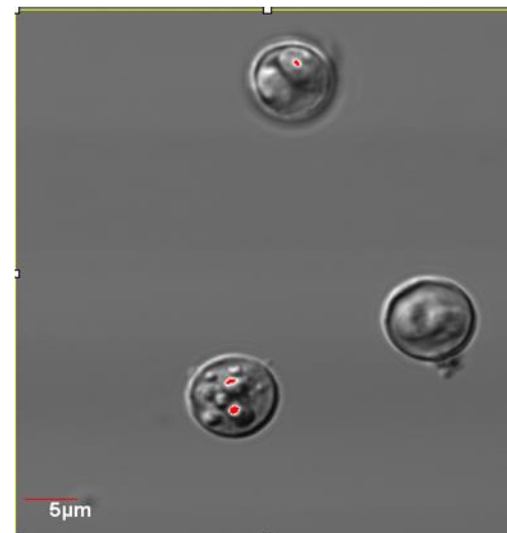
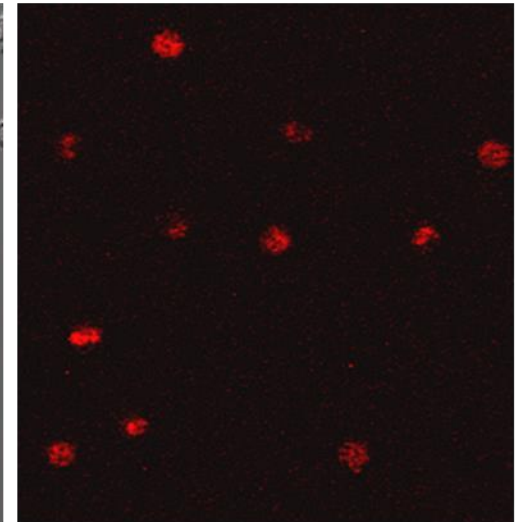
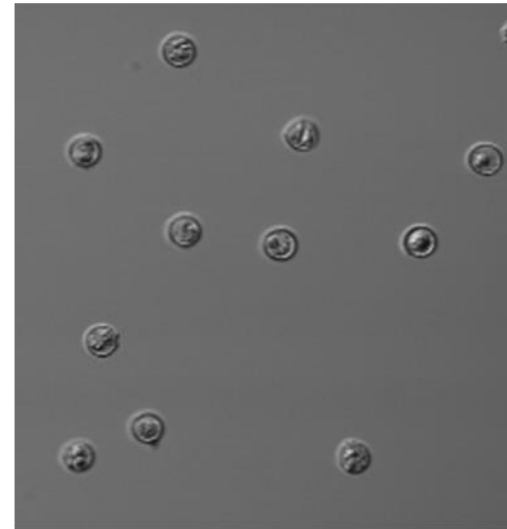


Assay Controls

**No Aptamer
(-)ve Control**



**WP2-1 + Cyclospora
(+)ve Control**



Conclusion: *Toxoplasma gondii* showed no positive signals with any of the aptamers.