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Dear Rizal,

I wish I was there to help sort things out since it would be easier if I could look at the microscope and see what is going on. Your strategy sounds right. It is not clear to me from the email whether you got step one to work. I will assume you did not.

Here are some answers to your questions:

- Yes, I got a 100X TIRF objective thinking that it would give us stronger focus and more intense light. Those were the main reasons for choosing it.

- We are using the TIRF objective to excite fluorescence, not to produce an image. For that purpose a tube lens should not be needed.

To provide you with more rationale for why the rest of the microscope was constructed the way it was, I note that the other objective, the one that produces the image, is also infinity corrected and we were able to get images with it without a tube lens.

Our microscope is somewhat unconventional in that we have a very long tube length that should approximate infinity and we do not have eyepiece optics between the camera and the tube. That is, the light that goes through the microscope objective goes directly to the camera without any intervening optics (except the filters). The rationale for this was that the light collection efficiency would be improved by minimizing the number of intervening optical elements from which light can be reflected and lost. Some infinity corrected objectives require a tube lens to eliminate chromic aberrations. It is my understanding that Nikon engineers its infinity corrected optics so that the chromic aberrations are corrected within the objective and not by the tube lens. In this case the infinity corrected lens should work just fine with our microscope configuration, the focal point will move out a little bit but that should be OK for us because it will give us a slightly larger working distance than the objective was designed for and, after all, we did get good images with it.

chromic aberration = chromatic aberrations?

”Chromatic aberration is an aberration of lenses, whereby light waves of different wavelengths are brought to focus at different locations along the optical axis. In the typical case of a simple thin lens, the focal length is shorter for blue wavelengths than it is for red ones.” (Murphy p. 334)

- Using the cheap camera might be a good idea for debugging the microscope. That way one would not have to worry about damaging the HAMAMATSU camera by inadvertently exposing it to too much light. Since for alignment you are using fluorescent beads, you should have plenty of signal for the cheap camera.

A thing to check into is if the TIRF objective needs a special high index immersion oil. I did not think that it did but it would be a good idea to check with Micron. Another thing to check

is what the working distance of the objective is and whether thin cover slips are needed. If the cover slip is too thick it may be that the laser light can only be focused within the cover slip and not on the surface to which the beads are attached. It would be good to try to arrange things so that within the sample volume the beads get attached to the surface that is closest to the TIRF objective, just in case the objective does not have a long enough working distance to reach the sample volume surface that is closest to the microscope objective.

If you use the cheap camera you could take out one of the two filters to give more light through-out. You could use a concentrated sample of beads so that there are many in a given field of view. Both of these should make the alignment process easier. Once you have had practice getting things to work this way you could go back to the original configuration and try to get things to work that way.

I can't remember the details of what the microscope looks like any more. Can you still open the black box to check if laser light is passing through both objectives? If this is the case, then the instrument should work because the laser light must be passing through the volume where the beads are located and so they should be excited. It might be good to remove the camera and take the filters out and see if you can get the laser light projected onto the ceiling of the room. That way we would know that fluorescence light should get to the camera.

Hope this helps,

Bernie Yurke