Optical Alignment for Fluorescence Measurements

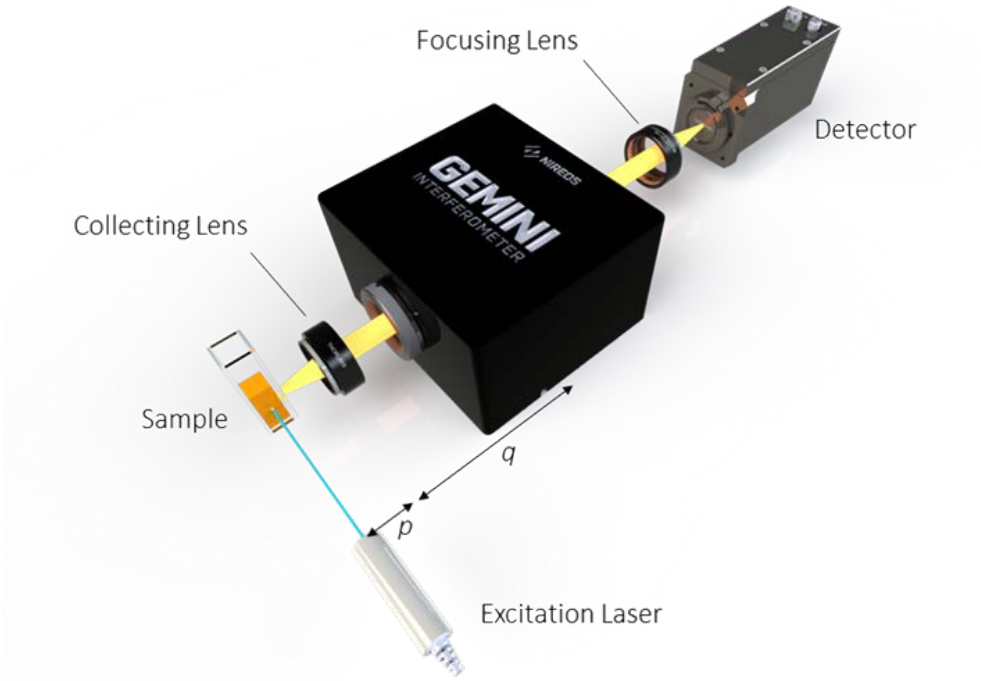

***FIGURE 1**: Experimental Setup showing how to use GEMINI in Fluorescence Experiments.*

Figure 1 shows an example of experimental setup in which GEMINI Interferometer coupled with a single-pixel detector (such as a SPAD or PMT) is used to measure the fluorescence signal emitted by a fluorescent sample.

The following procedure allows to properly align the optical elements together with GEMINI and the detector, in order to optimize the experimental conditions and obtain the best results.

STEP 1: Align the excitation laser, so that the beam (represented in blue in Figure 1) excites the fluorescent sample. If needed, a lens (not present in Figure 1) can be used to focus the light into the sample.

STEP 2: Collect the fluorescent signal (represented with a yellow beam in Figure 1) with a collecting lens, with focal length f. Approximately, as a first alignment, place the collecting lens at a distance p greater than f from the sample ($p=f+\delta$), so that the image of the sample is formed at a distance q from the collecting lens, following the equation: $1/p+1/q=1/f$.

STEP 3: Place GEMINI Interferometer after the collecting lens, in such a way that the image of the excited sample lies inside the interferometer. If possible, slightly adjust the position (distance p) of the collecting lens in order to maximize the

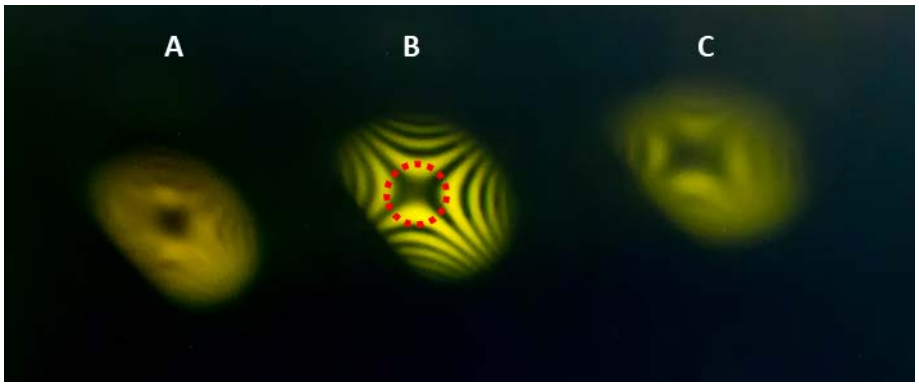

***FIGURE 2**: Three pictures of the light spot after the focusing lens, at different distances.*

throughput of light out of the GEMINI Interferometer.

STEP 4: Place a focusing lens after the Interferometer to focus the light inside your detector.

STEP 5: After the focusing lens, find the plane where spatial interference fringes have maximum contrast. In fact, after the focusing lens, the contrast of the fringes depends on the distance from the lens itself (please refer to Figure 2: in case A and C, the selected plane is not at the proper distance from the focusing lens: indeed, the interference fringes in these two cases show very poor contrast. Case B shows the best condition, where interference fringes show very good contrast). Place your detector so that its active area lies in this plane (case B in Figure 2).

NOTE: In order to maximize the contrast, you can use an aperture to select the central part of the spot (as an example, please refer to the red dotted circle in Figure 2(B)).