



Fig. 4. Measured enhancements of the excitation and emission intensity for spheres with a radius of (a) R = 80 nm and (b) R = 150 nm. The solid lines indicate the linear regression of the data points and the dashed lines represent the expected regression for light that is optimally concentrated to the smallest possible spot.

is defined as the ratio between the peak intensity of the focus and the average diffusive background intensity. The diffusive background intensity is determined by averaging the intensity at the probe sphere over 100 random realizations of the incoming wave front. The emission intensity enhancement is defined as the total emission when a focus is created divided by the average emission during the reference measurement. In Fig. 4 we have plotted the measured enhancements for (a) R=80 nm and (b) R=150 nm probe spheres. For the same probe size the emission enhancements $\eta^{\rm em}$ are proportional to $\eta^{\rm ex}$. The proportionality constant is clearly different for the two probe sizes. The number of control segments was varied to create a large spread in enhancements.

We now theoretically investigate the relation between $\eta^{\rm em}$ and $\eta^{\rm ex}$. The emission power caused by a focused excitation field is proportional to the focus intensity integrated over the probe sphere volume. The reference emission power scales with the volume of the probe sphere. On the contrary, the excitation enhancement is independent of the probe volume and is determined by dividing the peak intensity of the focus by the reference speckle intensity. The ratio of the enhancements for a probe sphere with radius R, C_R , is now given by

$$C_R \equiv \frac{\eta^{\rm em}}{\eta^{\rm ex}} = \frac{1}{V} \int_R \frac{I}{I_{\rm peak}} dV,$$
 (1)

where V is the volume of the probe sphere.

Assuming that plane polarized light is optimally focussed to the smallest spot area [19] in the center of the probe, we numerically calculate the overlap integral in Eq. 1. From the calculations we find that $C_{80}=0.77$ for the 80 nm spheres and $C_{150}=0.43$ for the 150 nm spheres (dashed lines in Fig. 4). These values are in good agreement with the experimental values C_{80} and C_{150} that we find from the linear regression of the data points, $C_{80}=0.72\pm0.07$ and $C_{150}=0.50\pm0.07$ (solid lines in Fig. 4). This data therefore support the conclusion that the light is being optimally concentrated.

In conclusion, we have focused light onto fluorescent probes hidden by a strongly scattering layer of zinc oxide by spatially shaping the phase of a light beam. We studied the shape and dimensions of the created focus. We found that the focus is optimally concentrated to a focus whose area is for certain within 68% of the smallest focal area physically possible. A study of the intensity enhancements of both the fluorescence and excitation, performed on different probe sizes, supports the conclusion of optimal light concentration.

The authors thank Ivo Vellekoop and Willem Vos for support and valuable discussions. We thank Timmo van der Beek for help with the angular resolved transmission measurements. A. P. Mosk is supported by a VIDI grant from NWO.

References

- P. Sebbah, "Waves and Imaging through Complex Media," (Kluwer Academic Publishers, 1999)
- A. Ishimaru, "Limitation on image resolution imposed by a random medium," Applied Optics 17, 348–352 (1978)
- D. Huang, E.A. Swanson, C.P. Lin, J.S. Schuman, W.G. Stinson, W. Chang, M.R. Hee, T. Flotte, K. Gregory, C.A. Puliafito, and J.G. Fujimoto, "Optical coherence tomography," Science 254, 1178-1180 (1991)
- J.M. Schmitt, "Optical coherence tomography (OCT): a review," IEEE Journal of Selected Topics in Quantum Electronics 5, 1205-1215 (1999)
- W. Denk, J.H. Strickler, and W.W. Webb, "Two-photon laser scanning fluorescence microscopy," Science 248, 73-76 (1990)
- F. Helmchen and W. Denk, "Deep tissue two-photon microscopy," Nature Methods 2, 932-940 (2005)
- E.N. Leith, and J. Upatnieks, "Holographic Imagery Through Diffusing Media," J. Opt. Soc. Am. 56, 523-523 (1966)
- Z. Yaqoob, D. Psaltis, M.S. Feld, and C. Yang, "Optical phase conjugation for turbidity suppression in biological samples," Nature Photonics 2, 110-115 (2008)