Optical laser beam scanner lens relay system

1. Introduction

Laser beam scanning is used most often by far in confocal microscopes. There are many ways by which a laser beam can be scanned across the back focal plane of an objective lens, but in general, the most popular ways utilise a tube lens and a scan lens. A beam scanning arrangement is placed in back focal plane of the scan lens, generally consisting of at least one mirror mounted on a galvanometer driver. As the mirror rotates, the angle of incidence of the resulting collimated beam varies at the back focal plane and this causes a diffraction-limited spot to move in the objective's focal plane
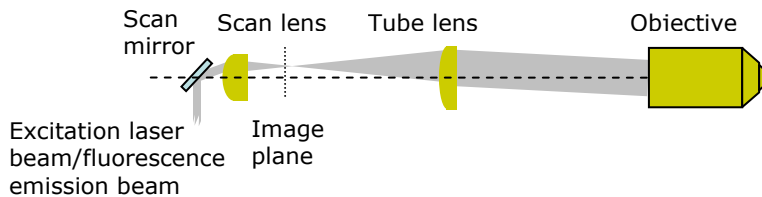

Figure 1. A standard microscope laser beam scanning optical arrangement

Such an arrangement is shown n Figure 1. A 160-200 mm focal length tube lens is typically used in conjunction with a short focal length scan lens, typically 25-35 mm focal length. Since the back aperture of most modern objectives is of the order of 15-25mm maximum, this dictates the laser beam diameter in this afocal arrangement; assuming 200 mm fl and 30 mm fl tube and scan lenses, the beam diameter should be 3 mm diameter for a 20 mm back aperture. A relatively short optical system is thus produced and very often microscope eyepieces are used 'in reverse' as scan lenses.

If a longer focal length scan lens is used, the laser beam diameter must be proportionately increased and the scan angle before the scan lens can be reduced. In some cases this can be advantageous because of the reduced scan angles, placing fewer constraints on the scanner mirror drive system. In practice, a scan lens of ~100 mm f.l. can be convenient as laser beam diameters of ~10 mm can be readily handled by small galvanometer scanners, as shown in Figure 2. In particular, when constructing time-resolved instruments, where potential reflections from optical components, mounting mechanics etc. need to be traced and eliminated, a longer, but still acceptable '4f' system is often desirable. Finally, when multibeam scanning arrangements are envisaged, wider laser beamlets are often desirable.
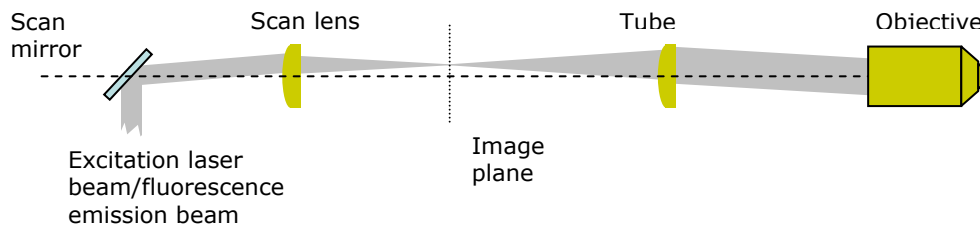

Figure 2. A longer microscope laser beam scanning optical arrangement

The scanner relay system

Figures 1 and 2 however do not take care of a fundamentally important issue: only a one-directional scan is catered for. In order to generate a two-dimensional scan, an additional scanner and mirror are required. Many designers use a close-coupled galvanometer/mirror system. In such systems,

neither the X or the Y scanners are optimally placed and the beam 'moves' in the objective back focal plane. We have never found such arrangements to be optimal when attempting to construct high quality microscopes, particularly when two-photon excitation is used. A superior method exploits the fact that one of the scanner mirrors can be optically relayed onto the surface of the other mirror. Such an optical relay system can be most optimally constructed using long focal length reflectors: any astigmatism introduced by one reflector can be compensated for by the other and achromatic operation can be readily obtained.

However, a long (or appropriately folded) optical system then results and this cannot be readily prototyped. Such arrangements are best left to commercial implementations of laser scanning instruments. We describe here a simpler system which can be constructed from Thorlabs (http://www.thorlabs.com/) cage system components in a linear fashion, as shown in Figure 3.
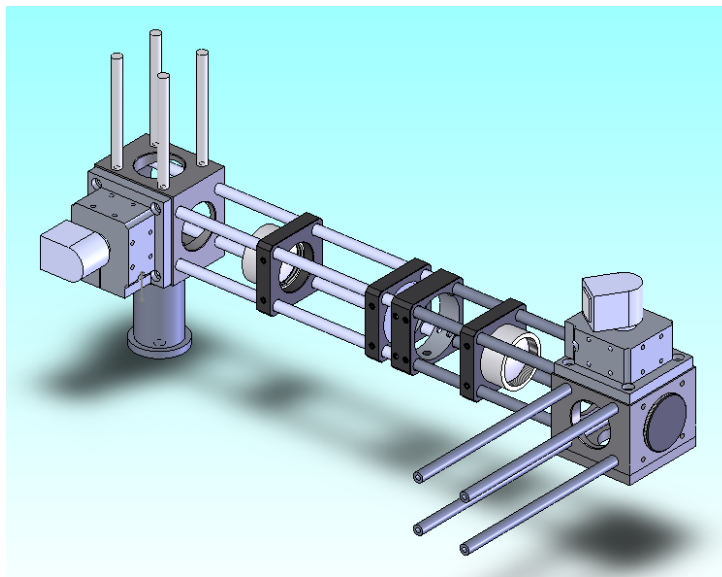

Figure 3. A galvanometer scanner lens relay system constructed form Thorlabs C6W cubes on the ends, CP02 / CP02T cage plates and SM1L05 lens tubes. A custom machined CP01 plate in the centre forms a slit and isolates the X and Y scanning systems. Plastic tubes (Thorlabs SC1L24) are held in the slit assembly and in dummy lens tubes (not shown in the SolidWorks model)

A lens-coupled system is inevitably chromatic to some extent and it is hard to ensure field flatness with standard, i.e. commonly available lenses. Nevertheless, a very good performance can be obtained with a little bit of care in the design. Even a very simple design using achromats is adequate, although significantly improved performance can be obtained if meniscus lenses are added. The general rule in optics is of course to use long focal length lenses and only use the central portion of the lens. An obvious way o achieve this is to reduce the scan angle as mush as possible and thus to utilize a relatively large laser beam diameter, as described above.

We present here the design that we use in our laser scanning microscopes. These have been primarily designed for time-resolved imaging applications where the relatively large, but not unduly large lens-lens distances allow reflections to be suitably removed through the use of baffles and beam stops where appropriate. We use this system in parallel with a widefield camera imaging system and thus adjust the image plane to have comparable dimensions to that of the camera used. This is a 2/3" chip size device and the image plane thus has a diagonal distance of 11 mm, though in practice a square, rather than rectangular image is used

The design has been modeled with Optics Lab (http://www.optics-lab.com/) ray-tracing software. This software package is extremely straightforward and intuitive and we find this convenient for prototype modeling. We first present, in Figure 4, the layout of the system and its on axis performance for three wavelengths 486 nm, 546 nm and 633 nm, with the design optimized in the green (546 nm). After the first scan lens, all rays 'fit' within the 16 μm diffraction-limited Airy disc, while after the scan lens (2 x 175 mm focal length achromats), the performance is somewhat worse,

but still close to the diffraction limit (~30 μm spot, rather than 25 μm spot). At any one wavelength, however, diffraction-limited performance can be obtained. These data are obtained with a laser beam diameter of 6 mm.
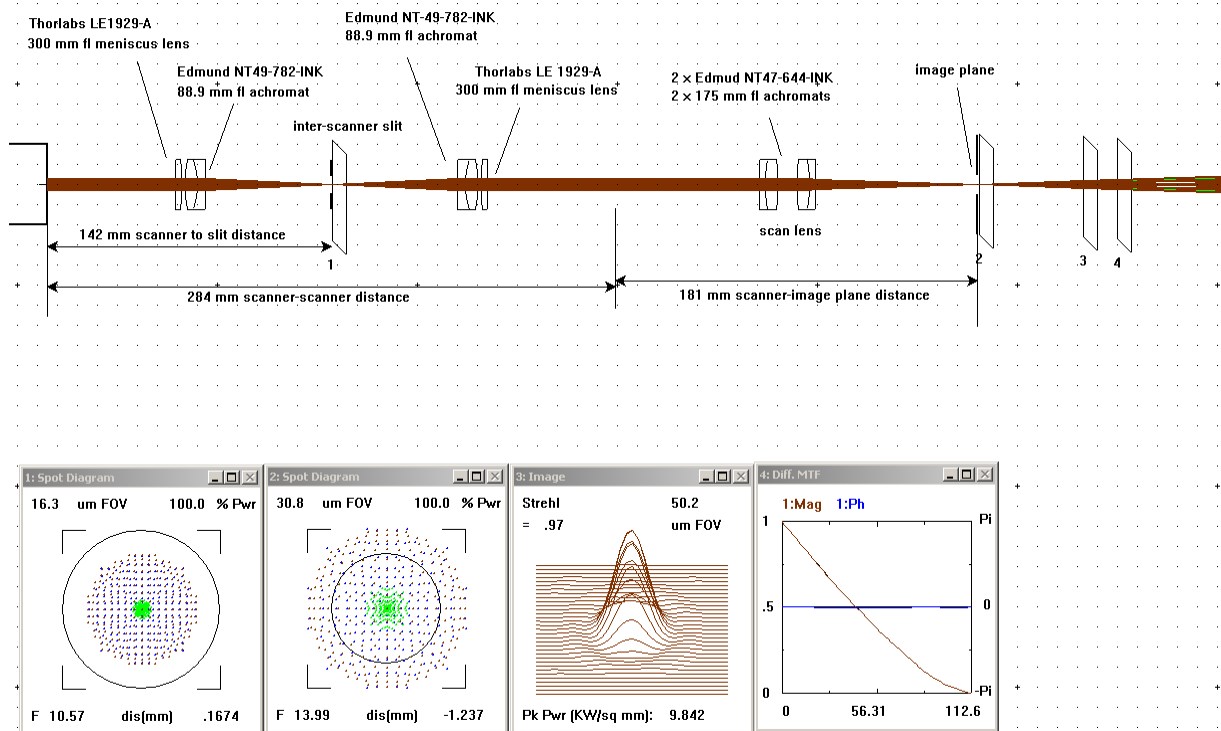

Figure 4. On-axis performance of scanner relay lens system and scan lens

We now increase the scan angle to 2.5 degrees, equivalent to the limit of the image dimension, and show this in Figure 5. The spot diameter, across the wavelength range is only slightly worse, and
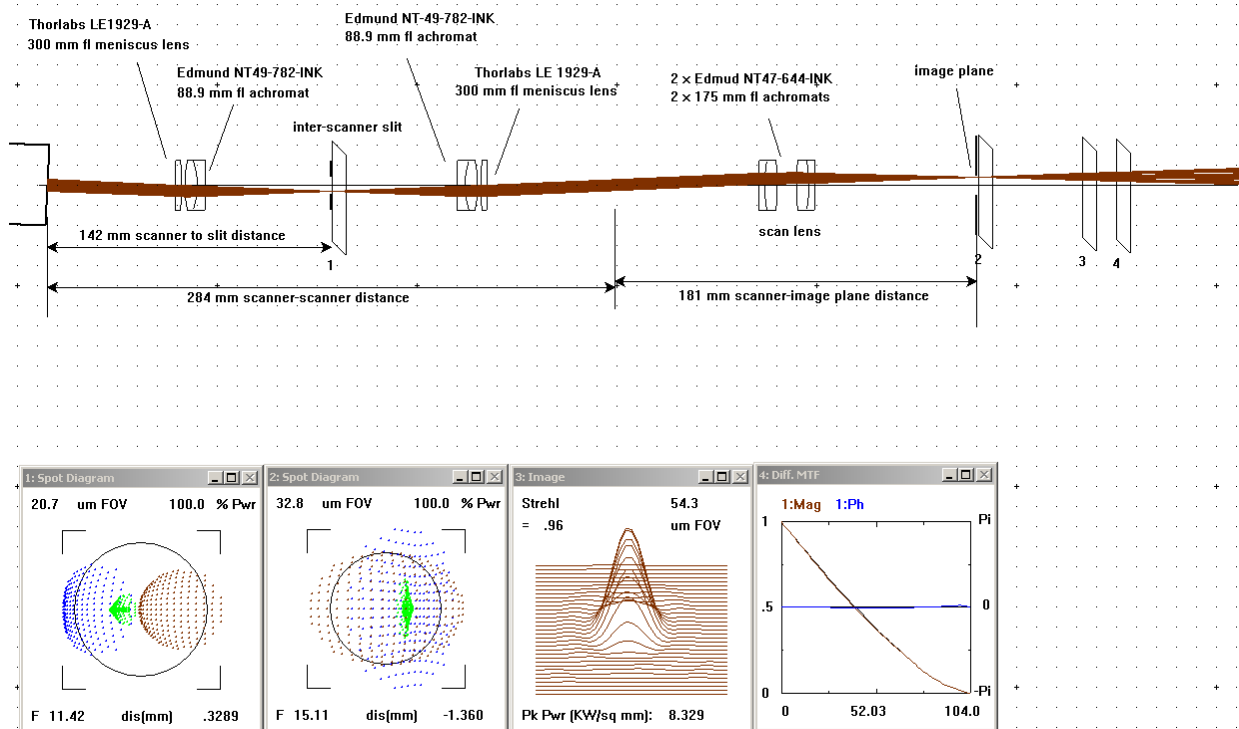

Figure 5. Performance of the scanner relay lens and scan lens system with a 2.5 degree scan angle, equivalent to a 9 mm diagonal image plane.

the modulation transfer function remains respectable at around 50 line pairs per mm for 50% contrast, close to the theoretical maximum. Although this looks perhaps worse than what could be tolerated, the data in Figure 5 refer to all wavelengths, so in Figure 6, we present data at individual wavelengths, indicating that the performance is actually pretty good!
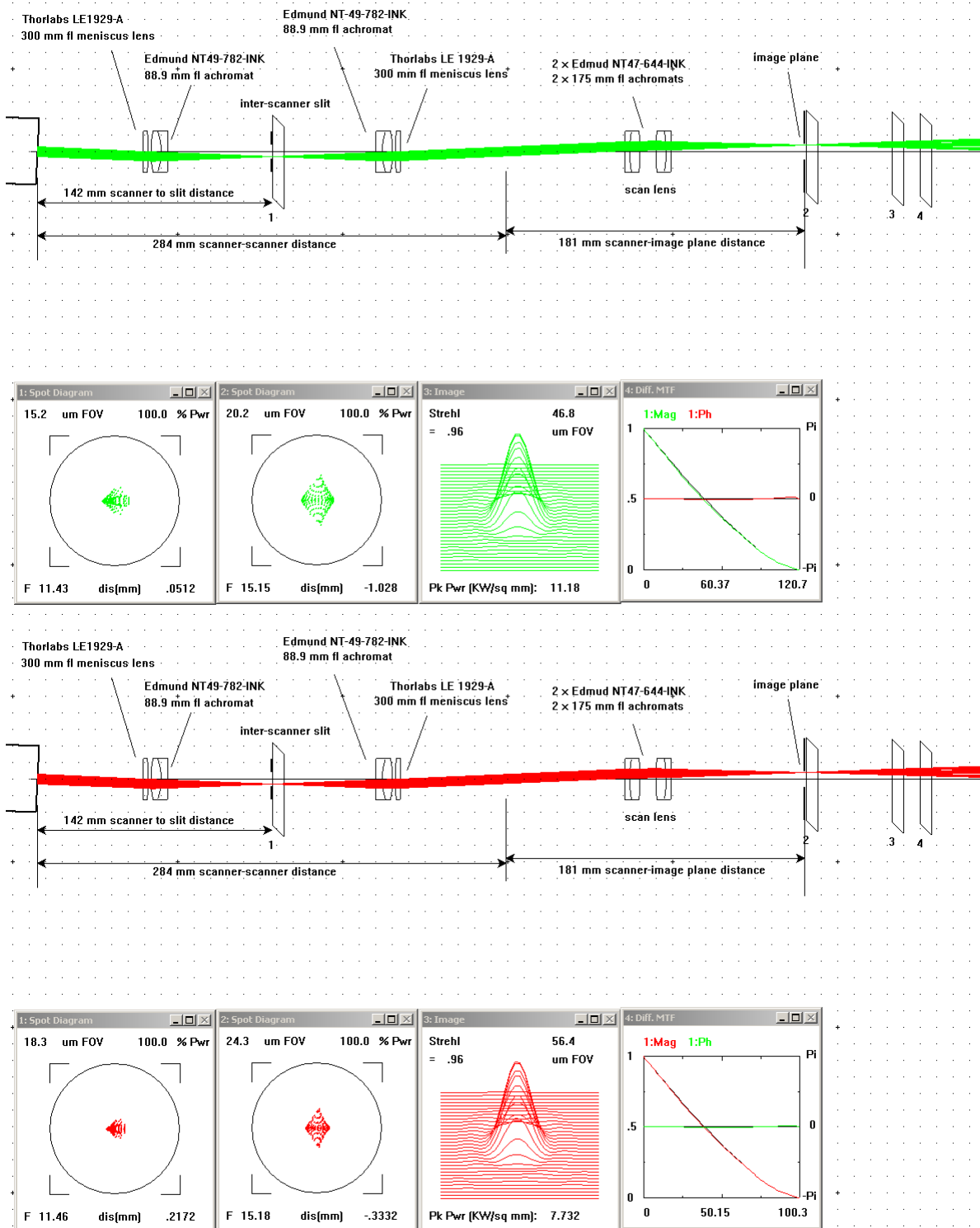

Figure 6. Performance of the scanner relay lens and scan lens system with a 2.5 degree scan angle, at red and green wavelengths, showing that diffraction-limited performance can be obtained with only a slight shift in the image plane (equivalent to a small change in focus position

The change in focus as function of wavelength is shown in Figure 7, for on axis (zero deflection) and a 2.5 degrees deflection, but this time a wider range of wavelengths is used (405 nm -656 nm).
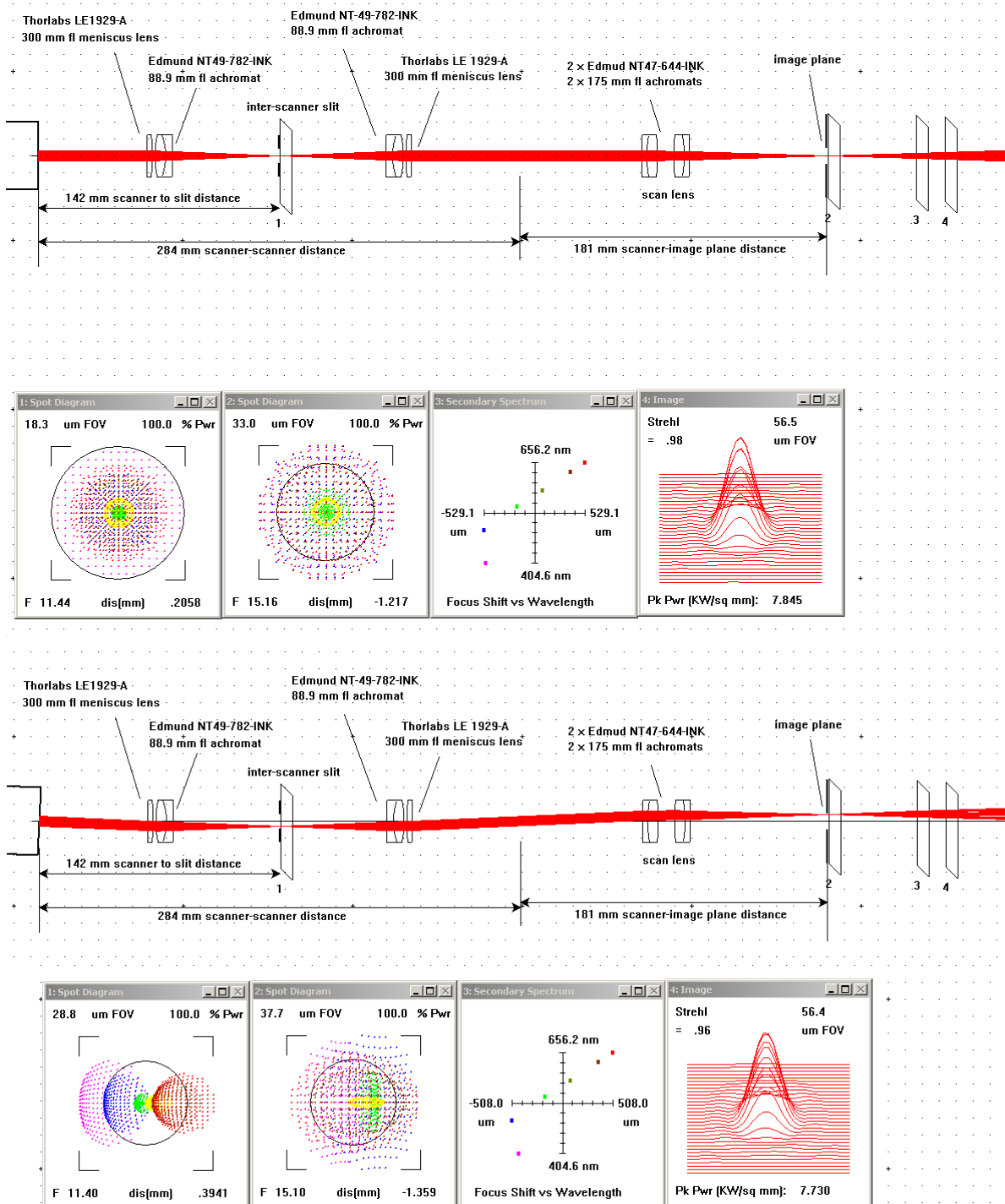
Figure 7. Focus shift as a function of wavelength for the scanner relay lens and scan lens arrangement, indicating that a very small (< 1 mm) of focus shift in the image plane is present. This is not ideal, but quite acceptable when working over a small range of wavelengths.

Finally, in Figure 8, we present the field curvature of the system. Some pincushion distortion is present, but this is of respectably low magnitude. Although not presented here, the performance of the optical system is similarly respectable 'in the other direction', i.e. as far as fluorescence emission light is concerned.
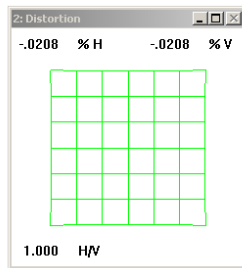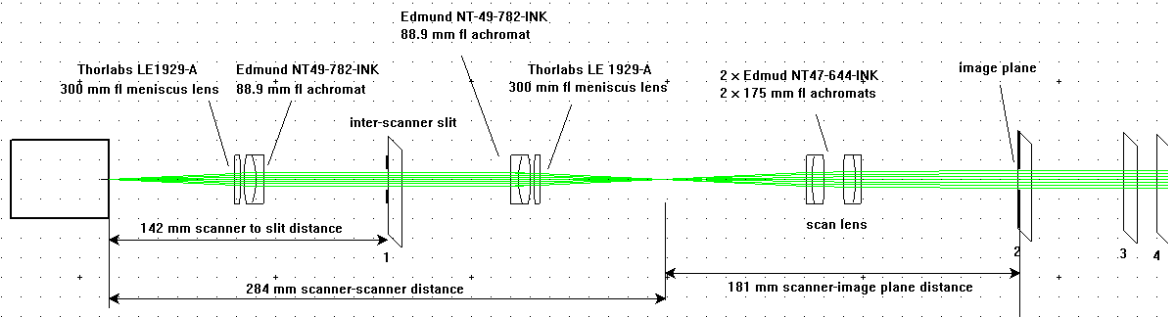
Figure 8. Field distortion performance of the system, showing that a very slight pincushion distortion is present

3. Performance

Of course, optical modeling is useful, but no amount of modeling can ever be a substitute for the actual performance of such a system. This of course depends on numerous other factors, such as microscope tube lens performance, objective performance, laser beam quality, dichromatic filter quality, etc. While we do not describe the other portions of the optical system here, we can show an example of an image acquired with this system, shown in Figure 9. This shows Human epithelial carcinoma cells (A431) stably expressing cdc42-GFP, imaged with a 20x, 0.75 na Nikon objective.
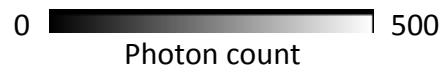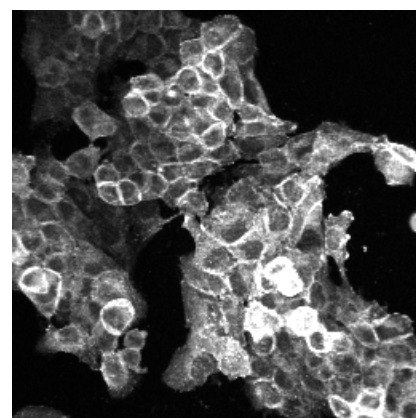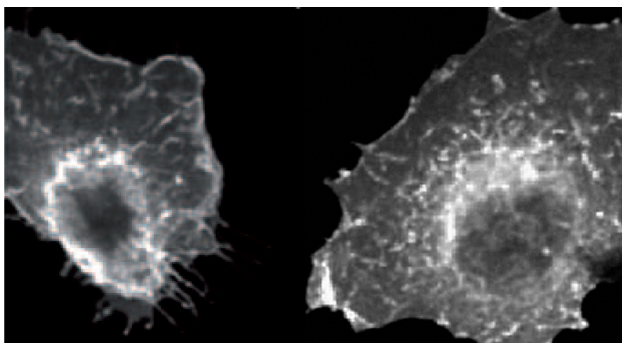

Figure 9. Example images obtained with the laser scanning system described here, showing GFP fluorescence from a human epithelial carcinoma cell line (right) and Cy2 emission from a breast cancer cell line.