Remote Focusing

An Exploration of Theory and Application to Microscopy

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1 Principles of Remote Focusing

1.1 Introduction

Remote focusing exploits variable dioptric power lenses and optics to change the position of a plane along the optical axis without physically translating it. This allows for some really exciting applications, such as electronically adjusting the focal plane within milliseconds when it may not otherwise be practical to do so physically. Introducing minimal aberration and magnification, it is an exciting tool in microscopy, where it allows fast, automated three dimensional imaging when implemented in the detection pathway of a microscope. In our lab, we also use remote focusing in the excitation light-path to dynamically control the focal plane of our illumination beam, enabling advanced fluorescent microscopy techniques such as TIRF and HIST. To understand why focusing works, I follow the derivation of Remote focusing from a Fourier Optics perspective [1] and a geometric optical perspective [2].

1.2 Remote Focusing and Transport-of-Intensity Fourier Optics

We assume our system is telemetric, which guarantees that refocusing avoids the introduction of additional phase-curvature across the field of view. This would result in the addition of variable magnification over the field of view and z-stack. This is crucial, as otherwise data processing would be unduly arduous and Under this assumption, we can model the propagation of the wavefunction u_0 modified by the free space transfer function H as follows.

$$u_{\Delta z}(x,y) = \mathcal{F}^{-1}\left[\mathcal{F}(u_0(x,y))H_{\Delta z}(u,v)\right] \tag{1}$$

In brackets, we see that we have taken the Fourier transform of the wave to find its corresponding frequency-space function, and then modify with the transfer function. In the paraxial approximation, the free space transfer function is known to be

$$H_{\Delta z}(u,\nu) = \exp\left[-i\pi\lambda\Delta z(u^2+\nu^2)\right]. \tag{2}$$

Replicating the phase modification of free space via a spatial light modulator (SLM) represents the angular spectrum method and is widely used in holographic reconstruction due to its ability to produce an image field which does not vary in size along the optical axis. However, there are many practical difficulties of working with an SLM, we instead utilize a electronically tunable lens (ETL). In Fourier optics, in the paraxial approximation a lens produces a transfer function of the form (CITE Goodman eq. 5-10)

$$t_l(x,y) = \exp\left[-i\frac{k}{2f}\left(x^2+y^2\right)\right]. \tag{3}$$

Since our ETL is in the spatial coordinates of the Fourier plane of the 4f system, it has a transfer function of

$$t_l(\xi,\eta) = \exp\left[\frac{-i\pi}{\lambda f_c}\left(\xi^2+\eta^2\right)\right]. \tag{4}$$

Notice that equation 4 is similar in form to the free space transfer function given in equation (2); by selecting a specific combined focal length f_c, we can do the equivalent of propogating the wave through free space some distance Δz. In our case, we use only a stand-alone ETL rather than a combined system, so $f_c = f_e$.. We may now express this in terms of (u,ν) and then compare it to our known free space transfer function $H_{\Delta z}$ above, then solve for Δz. Note that in this case we convert from Foruier spatial coordinates to frequency coordinates with CITE GOODMAN $(\xi,\eta) \to (\lambda f u, \lambda f \nu)$ to see

$$H_{\Delta z}(u,\nu) = t_l(\xi,\eta) \tag{5}$$

$$\exp\left[-i\pi\lambda\Delta z(u^2+\nu^2)\right] = \exp\left[\frac{-i\pi}{\lambda f_e}\left((\lambda f u)^2+(\lambda f\nu)^2\right)\right] \tag{6}$$

$$\Delta z = \frac{f^2}{f_e} \tag{7}$$

[1] Section 1.2 follows the derivation performed in by Zuo et al in [CITE].
[2] Section 1.3 follows the derivation performed by Qu and Hu [CITE].

1.3 Using Ray Optics to find Axial Scanning Range

Cite Qu, Hu! When centered at the focal plane of two relay lenses rather than at the back focal plane, ETL3 also causes minor demagnification at high dioptric power. To see why this is, we turn to another paper which analyzes the impact of a remote focusing objective in the detection pathway in more detail. We begin with the thin lens equation

$$\frac{1}{I} + \frac{1}{I'} = \frac{1}{f} \tag{8}$$

for f'_O is the focal length of the objective, I is the distance between the object and the objective and I' is the distance between the objective and the image. Now let us assume that we have an ETL at the back focal plane of our objective. This ETL produces an axial translation of the focal plane Δz. This means we may now write a system of two thin lens equations to model the light going from the object plane, through the objective, to the BFP where the ETL is, and then to the imaging plane.

$$\frac{1}{I - \Delta z} + \frac{1}{I'_1} = \frac{1}{f} \tag{9}$$

$$\frac{1}{f - I'_1} + \frac{1}{I'_2} = \frac{1}{f'_e} \tag{10}$$

The first thin lens equation models the system from the new object plane, which has been shifted by Δz from the old object plane via remote focusing by the ETL, through the objective, and to the image after the objective at a distance of I'_1. The second thin lens equation models the system from the image after the objective through the ETL of variable focal length f_e and to the second image after the ETL at a distance of I'_2. Due to the configuration of our system, the distance between the image after the objective and the objective is equal to the distance from the objective to the ETL at the back focal plane, which is the focal length, and the distance from ETL2 to the image after ETL2 where the camera is placed, so that

$$I' = I'_2 + f'_O. \tag{11}$$

Now, we solve this system of equations 8-11 via MATLAB to find Δz.

$$\Delta z = \frac{f^2}{f'_e} \tag{12}$$

This result is similar to the result we got above, but here our objective is in the back focal plane of the system and f is the objective's focal length, which is related to the magnification of our system.

Let us pivot to considering a 4F system with two relay lenses and an ETL in the plane conjugate to the back focal plane. In our system, ETL2 and ETL3 are at the focal plane of two relay lenses conjugate to the back focal plane rather than in the back focal plane itself, so this will model a system analogous to ours. In this case, we get a thin lens equations describing the transition from object through the objective to the image and from the intermediate image through the ETL to the detector

$$\frac{1}{I - \Delta z} + \frac{1}{I'_1} = \frac{1}{f} \tag{13}$$

$$\frac{1}{I' + f'_r - I'_1} + \frac{1}{I'_2} = \frac{1}{f'_r} \tag{14}$$

where we maintain the same variable convention and introduce I'_2 is the distance between the image after the first relay lens and the relay lens and f'_r is the focal length of the relay lenses. In this system, the ETL is a distance d_1 from the relay lens, we get

$$d_1 = I'_2 + f'_e. \tag{15}$$

The distance from the relay lens to the ETL is given by

$$d_1 = f'_r + \frac{f'^2_2}{M_0 f}. \tag{16}$$

Using MATLAB, we solve for Δz

$$\Delta z = \frac{f_r'^2}{M_0^2 f_e'} \tag{17}$$

where $M_0 = (f_O' - I)/f_O'$ is the magnification of our system.

While this configuration much less axial range than the back focal plane setup, but it offers key advantages. Theoretically, the dioptric power of the ETL does not affect the magnification of the system in either the back focal plane or conjugate to the back focal plane. In practice it does however, and also causes spatial distortions in the image real world conditions. These are very manageable in the relay lens setup, especially for low dioptric power. [CITE qu hu and bio guy] Furthermore, the relay lens setup allows us to use two relay lenses in a fluorescent light microscopy setup, with one to translate the excitation pathway and the other to translate the emission pathway.

2 Calibration

2.1 Introduction to VIEW-MOD Optical System

Our optical system [CITE VIEW-MOD] is designed for light sheet fluorescence microscopy, and has two paths which diverge at the dichroic mirror of a filter cube just after a shared objective. In our system, one remote focusing lens, ETL2, is responsible for remote focusing in the illumination light path, while the other remote focusing lens, ETL3, is responsible for focusing in the detection light path. In our field of research, it is important to be able to translate the focal plane of the light sheet by a set amount using ETL2, while ETL3 must be able to move the imaging plane to the same location. In order to use these devices as designed, we must therefore create a look up-table for each ETL, tying applied current to optical axis translation Δz.

In theory, both the remote focusing ETLs follow equation 19. Since we have $f = 125mm$ relay lenses, we get a predicted axial transfer range of $\Delta z = \frac{15625mm^2}{M^2 f_{ETL}}$. We use ETLs with a diopric range of [-10, 10] giving a predicted range $\Delta z = [-156.25, 156.25]mm/M^2$. This predicts a range of $\pm 391\mu m$ for a 20X objective and $\pm 43.4\mu m$ for a 60X objective. However, we may control only current applied to the ETL, which controls dioptric power. Dioptric power is dependent on both current and temperature, which in turn is itself dependent on current. The non-ideal behavior of the ETL compounds with the non-ideal behavior of optics so that in practice, experimental behavior differ significantly theoretical predictions. To ensure accurate axial control, we calibrate our ETLs under experimental conditions.

Going forward, it will be important to differentiate between the excitation focal plane, the sample plane, and the detection focal plane. The *excitation* focal plane is the plane in which the beam waist lies, and is translated via altering the dioptric power of ETL2 or physically moving the objective. The sample plane is the physical location of the sample's surface, and is static for any given experiment. The *detection* focal plane is the plane which is imaged on the CCD, and is translated via altering the dioptric power of ETL3 or physically moving the objective.

2.2 ETL2 Calibration

ETL2 is in the excitation pathway of Hestia. Using either a spot or light sheet, we will first focus the excitation beam into the sample plane via objective height adjustment and ETL2. We use a slide coated generously with fluorescent 200nm beads as our sample.

We will use μManager to move the microscope objective upwards in the Z direction in known discrete steps. We then bring this image into focus in the camera via ETL3 to adjust for the objective displacement and refocus the image. We then minimize the width of a Gaussian light-sheet by using ETL2 to translate the excitation focal plane back into the sample plane. Because we are compensating for a known displacement of the objective via ETL2, we can link the current value of ETL to focal-plane translation. Taking many of these steps allows us to map the relationship between ETL2 current and Δz_{ex}.

2.3 ETL3 Calibration

ETL3 is in the detection plane of the microscope. To calibrate it, we follow much the same procedure we did for ETL2, but rather than involving the excitation pathway we simplify things using the built-in transmission illumination lamp and a calibration grid. We translate the objective by a known step, then compensating for the output with ETL3, moving the detection focal plane back into the sample plane and recording the current necessary to do so. In this way we can create a map of the relationship between ETL3 current and Δz_{em}.

2.4 Light-Sheet Characterization

In this experiment, we use our ETLs to decouple the emission and excitation focal planes and use this image and characterize the profile of a light sheet. First, we use ETL2 to focus the light sheet in the sample plane, where we again use a slide with 200nm beads for our sample. Then we displace the objective by known increments, translating both the emission and excitation focal planes by Δz . Then we beads back into focus using ETL3, moving the emission focal plane back into the sample plane. We then image the beam at some distance from its beam waist δZ. After doing this for a variety of objective displacements, we analyze the images to determine the beam waist as a function of axial displacement, $\omega(z)$.

3 Results

3.1 ETL1

3.2 ETL2

3.3 Light Sheet Characterization

4 Junk!

In our 4F setup, we use optical lenses are the relay lenses of our 4F system, which have $f = 125mm$. Plugging this into equation 8 and 10, in a system without magnification we get $\Delta z = \frac{15625mm^2}{f_{ETL}}$. Our ETL (Optotune 10-40) has an effective focal power range of -10 to 10 diopters. Dioptric power is given by $f_{ETL} = \frac{1m}{D}$ and therefore for us ranges from $f_{ETL} = (-\infty, -10] \cup [100, \infty)$ millimeters. This gives us a z-translation range of

$$\Delta z = [-156.25, 156.25]mm. \tag{18}$$

$$\frac{\Delta z}{M^2}, \tag{19}$$

so for a 60X objective we get $\Delta f = [-43.4, 43.4]\mu m$. Likewise, for a 40X objective, we get $[-97.7, 97.7]\mu m$, and for a 20X objective, we get $[-391, 391\mu m]$. Whg

Note that the authors of HIGH SPEED TRANSPORT CITE give

$$f_e = \frac{f_{ETL} f_{OL}}{f_{ETL} + f_{OL} - d} \tag{20}$$

Specifically, the light passes through a flat pane of glass before hitting the ETL, so the focal length of our optical lens goes to infinity. Since the optical lens focal length blows up to infinity, it dominates the denominator and cancels with itself in the numerator so that

$$\lim_{f_{OL} \to \infty} f_c = f_{ETL} \tag{21}$$

which simplifies our calculations significantly. But we have yet to account for the magnification of our system, which we do below. CITE Grewe, B.F., et al., Fast two-layer two-photon imaging of neuronal cell populations using an electrically tunable lens. Biomed Opt Express, 2011. 2(7): p. 2035-46.