1 Introduction

Scientists continue to discover new biological mechanisms in human and animal health with *in vivo* microscopy. To observe dynamic phenomena as they occur, intact and thick samples are often needed for observation. Within these living tissues, microscopists must be able to follow specific trajectories in a 3D volume, track/stabilize features of interest, and minimize agitation of the sample during imaging. Varifocus elements allow for fast electronic focusing without translation of the sample stage or objective lens, thereby enabling dynamic and agile imaging within thick samples. This Spotlight introduces miniature varifocus elements and presents the optical equations needed to integrate such elements with microscopes. It reviews the current technological capabilities and limitations of varifocus elements, such as liquid crystal devices, liquid lenses, vertical displacement microlenses, and deformable membrane mirrors. A discussion of varifocus elements demonstrated in widefield microscopes, confocal microscopes, two-photon microscopes, and optical coherence tomography is included.

Chapter 1 begins by discussing the motivation for varifocus elements in *in vivo* microscopy. It then provides an overview of the most common types of varifocus elements.

1.1 Motivation

The ability to observe dynamic biological phenomena provides an avenue for understanding basic mechanisms that play vital roles in animal and human health. Specifically, scientists desire the ability to observe dynamic phenomena that can be viewed below a tissue's surface, such as polarity, environmental relaxation, drug delivery, ion channels, and electrostatic potentials in cell membranes. Furthermore, accurate detection of cancerous tissue without a physically invasive biopsy is an area of concern,[1] as the naked eye and simple cameras can only detect surface views of most tissues.

Confocal microscopes and other imaging instruments provide vital information for disease diagnosis and biological observation by enabling subsurface, high-resolution imaging. In most of these instruments, motors or linear actuators translate a sample stage or objective lens to change the depth of the image plane within the specimen. Point-by-point scanning is a common technique that acquires several *x*-*y* planes and stitches them together to form a three-dimensional (3-D) (*x*-*y*-*z*) stack (Fig. 1). For *in vivo* microscopy, this process slows the speed of image acquisition, overexposes fluorescent dyes (leading to photobleaching and photodamage), and can agitate or damage sensitive samples. The *x*-*y* scanning approach is most often performed within the optical train, or optical assembly, inside the instrument and does not rely on physical translation of the sample stage. A purely optical technique for performing focus control will prevent agitation of sensitive samples from stage or objective lens movement while allowing rapid image acquisition.