which sharpness is calculated, sharpness for a zone may be represented by EQUATION 2:

$$Sharpness = \Sigma_{i=0}^{n-1} \Sigma_{j=0}^{m-k-1} \left[\left(I_{i,j} - I_{i,j+k} \right)^2 \right] \quad \text{EQUATION 2}$$

~~$Sharpness = \Sigma_{i=0}^{n-1} \Sigma_{j=0}^{m-k-1} (1_{i,j} - 1_{i,j+k})^2 \Sigma_{j=0}^{m-k-1} \left[\left(I_{i,j} - I_{i,j+k} \right)^2 \right]$~~

~~EQUATION 2~~

where k is an integer between or equal to 1 and 5. For this embodiment, z = 1 (only one zone), although, in other embodiments, as further discussed elsewhere herein, more than one zone may be used in connection with the system described herein. Other sharpness metrics and algorithms may also be used in connection with the system described herein. As the XY moving stage 130 is moving along the y-axis, the system acquires sharpness information for the current zone in the focus frame 306, which information is used to determine a best focus position.

FIG. 9 is a schematic illustration 350 showing an example of a sharpness profile, produced from moving through focus positions, including a sharpness response curve 360 and contrast error signal 370 for each sharpness response at multiple points that are sampled by the dither focusing optics according to an embodiment of the system described herein. Plot 360 shows dither lens amplitude in micrometers in the x-axis and sharpness units along the y-axis. As illustrated, the dither lens motion may be centered at representative positions A, B, C, D and E; however, is it noted that the computations described herein may be applied to each of the points on the sharpness curve. The sharpness response produced from the focus sensor 160, for a half cycle of the dither lens sinusoid, when motion of the dither lens is centered at each of the positions A, B, C, D and E is shown, respectively, in the waveform plots 361-365.

As discussed herein, the dither lens may be vibrated at 60 Hz at approximately 300 microns peak-to-peak (p-t-p) amplitude. This produces a change in focus as seen by the focus sensor of about +/- 5 microns at the tissue. Best focus can be measured by the focus sensor by computing sharpness at each focus frame. This calculation may be done in the camera's FPGA. Therefore while the dither lens is vibrating at 60 Hz, 20 sharpness metrics may be computed per dither cycle (1200 sharpness calculations per second). Characteristic waveforms 361-365 are measured depending on the position of the microscope objective relative to best focus. For example, at best focus (position C) the dither lens samples either side of the sharpness response and produces sine wave (waveform 363) at two times the frequency of the dither vibration. Point 'a' at a sine wave trough, point 'b' at the