Theoretical and experimental demonstration of resolution beyond the Rayleigh limit by FINCH fluorescence microscopic imaging

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Abstract: Fresnel Incoherent Correlation Holography (FINCH) enables holograms to be recorded from incoherent light with just a digital camera and spatial light modulator. We previously described its application to general three dimensional incoherent imaging and specifically to fluorescence microscopy, wherein one complex hologram contains the three dimensional information in the field of view, obviating the need for scanning or serial sectioning. We have now further analyzed FINCH in view of linear system theory and in comparison to conventional coherent and incoherent two dimensional imaging systems. We demonstrate, theoretically and experimentally, improved resolution by FINCH, when compared to conventional imaging.

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References and links


1. Introduction

Digital coherent holography has unique advantages for many imaging applications. In some applications the recorded holograms contain three dimensional (3D) information of the observed scene [1], in others the holograms are capable of imaging phase objects [2,3]. Holography also enables implementing super resolution techniques [4] and even makes it possible to image objects covered by a scattering medium [5]. Because of these advantages, digital holography has become important in optical microscopy. Examples of utilizing digital holography as the basis for optical microscopes are the recently published studies of lensless compact holography-based microscopes [6–8]. Another example of using digital holography in microscopy is the holographic coherent anti-Stokes Raman microscope [9]. In the present study we extend our investigation of Fresnel Incoherent Correlation Holography (FINCH), a way to utilize holography with incoherent light, and which is another example of using digital holography in microscopy.

Our first report of FINCH published in 2007 [10] was about a method to capture digital holograms of 3D objects illuminated incoherently. The setup of FINCH is simple and only includes a collimation lens (objective in case of a microscope), a spatial light modulator (SLM) and a digital camera (CCD or CMOS). The principle of operation is also simple; incoherent light emitted from each point in the object being imaged is split by a diffractive element displayed on the SLM into two beams that interfere with each other. The camera records the entire interference pattern of all the beam pairs emitted from every object point, creating a hologram. Typically three holograms, each with a different phase constant in the pattern of the diffractive element, are recorded sequentially and are superposed in order to eliminate the unnecessary parts (the bias and the twin image) from the reconstructed scene. The resulting complex-valued Fresnel hologram of the 3D scene is then reconstructed on the computer screen by the standard Fresnel back propagation algorithm [11]. Unlike other techniques of incoherent digital holography, like scanning holography [12], or multiple view projection holography [13], FINCH is a non-scanning and motionless method of capturing holograms. Acquiring only three holograms is enough to reconstruct the entire 3D observed scene such that at every depth along the z-axis every object is in focus in its image plane. Our group has been involved with several works on this topic, including color holography [14], two studies on FINCH based microscopes [15,16], a method to suppress the noise of FINCH [17] and two works on FINCH operating in a synthetic aperture mode [18,19]. FINCH is a method of incoherent holography that can operate with a wide variety of light sources besides laser light. Because of this flexibility to practice high resolution holography with FINCH, it can be used to implement holographic applications which could not be realized in the past because they were limited by the need for coherent laser-light [1–5]. In this study we
theoretically analyze and experimentally demonstrate additional properties of FINCH relating to resolution.

Recently two other research groups reported studies about FINCH [20,21]. In one publication [20], the authors investigated the influence of the degree of spatial coherence of light on the quality of the reconstructed 3D profiles in FINCH. In the other publication [21], the authors proposed the conditions for optimal resolution with FINCH. They concluded that resolution in FINCH imaging cannot exceed that of a classical imaging system. In this report we present theoretical and experimental data that differs with their conclusions and we show that indeed, FINCH imaging can exceed standard optical imaging system resolution. In the present study we bring a more complete analysis of FINCH as an imaging system. Particularly, we address the question of which of the systems, FINCH or a conventional glass-lens-based imaging system, can resolve better. There is not an obvious answer to this question because FINCH has unique properties that do not exist in conventional optical imaging systems; on one hand, the FINCH hologram is recorded by incoherent illumination, but on the other hand this hologram is reconstructed by the Fresnel back-propagation process, exactly as is done with a typical coherent Fresnel hologram. So the question is whether FINCH behaves like a coherent or incoherent system, or whether it has its own unique behavior. Knowing that the difference between coherent and incoherent imaging systems is expressed, among others, by their different modulation transfer function (MTF), the more specific question is what kind of MTF characterizes FINCH. Does FINCH have an MTF of a coherent or incoherent imaging system, or does it have its own typical MTF? The answer to this last question can determine the answer to the resolution question. In this report we analyze the transverse resolution of FINCH and show here, both theoretically and experimentally, that FINCH imaging significantly exceeds the resolution of a conventional microscope optical imaging system.

Fig. 1. Comparisons of the optical configuration for (a) FINCH with only one diffractive lens and (b) A regular optical imaging system with the same parameters used in (a).
2. FINCH system analysis

2.1. Mathematical analysis

FINCH, in the present model, creates holograms in a single channel system as a result of interference between two waves originating from every object point located in front of a collimating lens. The following analysis refers to the system scheme shown in Fig. 1(a), where it is assumed that the object is an infinitesimal point and therefore the result of this analysis is considered as a point spread function (PSF). For simplicity, we assume that the object point is located at \( \mathbf{r}_g = (x_g,y_g) \) on the front focal plane of the collimating lens \( L_1 \) (an objective lens in the case of an infinity corrected microscope system). A more general and extensive analysis is given in Ref [16], but for the purpose of the present study the simpler case is enough to learn about the features of FINCH. For an infinitesimal object point with the complex amplitude

\[
I_h(u, v) = \left| \sqrt{I_o} C(\mathbf{r}_g) L(\mathbf{r}_o) Q\left(\frac{1}{f_o}\right) Q\left(-\frac{1}{f_o}\right) \right| \left( B + B' \exp(i\theta) \right) Q\left(\frac{1}{f_d}\right) P(R_h) \right|^2,
\]

where \( f_o \) is the focal length of lens \( L_1 \), \( d \) is the distance between the lens \( L_1 \) and the SLM, \( z_h \) is the distance between the SLM and the camera, \( \mathbf{r} = (u, v) \) are the coordinates of the camera plane and \( B, B' \) are constants. For the sake of shortening, the quadratic phase function is designated by the function \( Q \), such that \( Q(b) = \exp\left[ i\pi b/\lambda \left( x^2 + y^2 \right) \right] \), where \( \lambda \) is the central wavelength of the light. \( L \) denotes the linear phase function, such that \( L(\mathbf{r}) = \exp\left[ i2\pi\lambda/\lambda \left( s_x, x + s_y, y \right) \right] \), and \( C(\mathbf{r}_g) \) is a complex constant dependent on the source point's location. The function \( P(R_h) \) stands for the limiting aperture of the system, where it is assumed that the aperture is a clear disk of radius \( R_h \) determined by the overlap area of the two interfering beams on the camera plane. The expression in the square brackets of Eq. (1) describes the transparency of the SLM. This transparency is a combination of a constant valued mask with a diffractive positive spherical lens of focal length \( f_o \). In the past we presented two methods to display these two masks on the same SLM. The older, and less efficient, method is to randomly allocate half of the SLM pixels to each of the two masks [10,14]. Lately [16] we have learned that a better way is by use of a positive lens mask over the whole SLM and light with two mutually orthogonal polarization components, one of which is parallel to the polarization of the SLM and the other which is orthogonal to it, so that the interference happens between the projections of each polarization component of the light beam on the crossing angle between the two orthogonal polarizations. The angle \( \theta \) is one of the three angles used in the phase shift procedure in order to eliminate the bias term and the twin image from the final hologram [10,14–18,22]. The asterisk in Eq. (1) denotes a two dimensional convolution. The explanation of Eq. (1) is as follows: the four left-most terms

\[
\sqrt{I_o} C(\mathbf{r}_g) L(-\mathbf{r}_g/f_o) Q(1/f_o)
\]

describe the point source wave as is seen from the plane of lens \( L_1 \). This wave is multiplied by the lens \( L_1 \) [multiplied by \( Q(-1/f_o) \)], propagates a distance \( d \) [convolved with \( Q(1/d) \)] and meets the SLM where its transparency is in the square brackets of Eq. (1). Beyond the SLM there are two different beams propagating an additional distance \( z_h \) till the camera [convolved with \( Q(1/z_h) \)]. On the camera detector, only the area of the beam overlap, denoted by the area of \( P(R_h) \), is considered as part of the hologram. Finally, the magnitude of the interference is squared to yield the intensity distribution of the recorded hologram. It is easy to see from Fig. 1(a) and by calculating Eq. (1), that as long as the source
point is located on the front focal plane of \( L_1 \), the interference occurs between a plane and a spherical (in the paraxial approximation) wave.

Three holograms of the form of Eq. (1) with three different values of the angle \( \theta \) are recorded and superposed in order to obtain a complex hologram of the object point, given by,

\[
H(\vec{p}) = C' I_r P(R_r) L \left( \frac{\vec{r}_r}{z_r} \right) Q \left( \frac{1}{z_r} \right),
\]

(2)

where \( C' \) is a constant and \( z_r \) is the reconstruction distance from the hologram plane to the image plane calculated to be,

\[
z_r = \pm |z_h - f_o|.
\]

(3)

The \( \pm \) indicates that there are twin possible reconstructed images although only one of them is chosen to be reconstructed, as desired. \( \vec{r}_r \) is the transverse location of the reconstructed image point calculated to be,

\[
\vec{r}_r = \left( x_r, y_r \right) = \frac{z_h}{f_o} \vec{r}_o.
\]

(4)

The precise way by which the results of Eqs. (2)-(4) are calculated from Eq. (1) is described in Ref. [18], and therefore we save the detailed algebra from being repeated herein. From Eq. (4) it is clear that the transverse magnification is \( M_T = z_h/f_o \). The PSF of the system is obtained by reconstructing digitally the Fresnel hologram given in Eq. (2) at a distance \( z_r \) from the hologram plane. The expression of the hologram in Eq. (2) contains a transparency of a positive lens with focal distance \( z_r \) and hence, according to Fourier optics theory [11], the reconstructed image is,

\[
h_r(\vec{r}) = C'' I_r \nu \left[ \frac{1}{\lambda z_r} \right] \mathcal{F} \left\{ L \left( \frac{\vec{r}}{z_r} \right) P(R_r) \right\}
\]

\[
= C'' I_r \text{Jinc} \left( \frac{2\pi R_h}{\lambda z_r} \sqrt{(x-M_r x_r)^2 + (y-M_r y_r)^2} \right).
\]

(5)

where \( C'' \) is a constant, \( \mathcal{F} \) denotes Fourier transform, \( \nu \) is the scaling operator such that \( \nu[a f(x)] = f(ax) \), \( \vec{r} = (x, y) \) are the coordinates of the reconstruction plane, \( \text{Jinc} \) is defined as \( \text{Jinc}(r) = J_1(r)/r \) and \( J_1(r) \) is the Bessel function of the first kind, of order one.

Equation (5) describes the two dimensional PSF of FINCH. Recalling that the object is a collection of infinitesimal incoherent light points which cannot interfere with each other, we realize that each independent object point is imaged to an image of the form of Eq. (5). The complete image of many object points is a convolution integral between the object denoted by intensity distribution \( I_s(\vec{r}) \) with the PSF shown in Eq. (5), as follows,

\[
I_s^L(\vec{r}) = I_s(\vec{r}) * h_r(\vec{r}).
\]

(6)

Equation (6) indicates that FINCH is a linear invariant system for the quantity of light intensity. However, since \( h_r \) is in general a complex valued function, \( I_s^L \) might be a complex valued function as well. This observation does not contradict any physical law because the reconstruction is done digitally by the numerical algorithm of the Fresnel back propagation [11]. The superscript \( L \) is added to the intensity obtained by Eq. (6) in order to distinguish it from the non-linear reconstruction discussed next.
In case the hologram is reconstructed optically by illuminating the hologram with a coherent plane wave, the output intensity is

\[ I^N (\tau) = |I, (\tau) * h_F (\tau)|^2. \]  

\( I^N \) denotes intensity of the optical reconstruction, or non-linear digital reconstruction as is demonstrated in the experimental part of this study. This image is not linear in relation to the gray levels of \( I, (\tau) \), but in some cases, for instance, binary objects whose images are not distorted by the non-linear operation, \( I^N \) is preferred over \( I^L \) because the side lobes of \( h_F \) are suppressed by the square operation, which results in improved image contrast.

The width of the PSF in every imaging system determines the resolution of the system. The width of the PSF is chosen herein as the diameter of the circle created by the first zero of the \( \text{Jinc} \) function of Eq. (5). This diameter remains the same for both the linear and non-linear reconstructions, and is equal to \( 1.22 \lambda z / R_H \). According to Eq. (3), \( z = |z_h f_d / R \) and therefore, based on a simple geometrical consideration, the radius of the hologram, which is the radius of the overlap area between the plane and the spherical beams, is,

\[ R_H = \begin{cases} R_o \frac{|z_h - f_d|}{f_d} & f_d \geq \frac{z_h}{2} \\ R_o & \text{Otherwise} \end{cases}, \]  

where \( R_o \) is the radius of the smallest aperture in the system up to, and including, the SLM. For \( f_d < z_h / 2 \) the projection of the spherical wave exceeds beyond the plane wave projection and therefore the radius of the overlap remains as \( R_o \). Consequently, the width of the PSF for the regime of \( f_d \geq z_h / 2 \) is

\[ \Delta = \frac{1.22 \lambda z_h}{R_H} = \frac{1.22 \lambda |z_h - f_d|}{R_o |z_h - f_d|} f_d = \frac{1.22 \lambda f_d}{R_o}. \]  

This PSF has exactly the size one would expect to see in the output of a regular imaging system shown in Fig. 1(b). At first glance, one might conclude that since the two systems have the same PSF, with the same width, their resolving power is the same. However Eq. (4) indicates that the location of the image point in the output plane of FINCH is at \( \tilde{r}_d z_h / f_d \). This is in general different than the location of the image point of the imaging system of Fig. 1(b), which is \( f_d \). In other words, if the two systems observe the same two object points, the size of all the image points in the two systems is the same, but the gap between the two image points differs between the two compared systems. The two point gap of FINCH and of the regular imaging system differ by the ratio of \( z_h / f_d \). Recalling that resolution is related to the gap between image points, as is manifested by the well known Rayleigh criterion, we realize that if \( z_h / f_d > 1 \), then FINCH can resolve better than a regular system. This is because in FINCH, the gap between every two image points is larger by a factor of \( z_h / f_d \) compared to the two point gap of a regular imaging system with the same numerical aperture. Moreover, increasing the ratio \( z_h / f_d \) in FINCH increases the resolution, where the maximum resolving power is achieved for the ratio \( z_h / f_d = 2 \). Beyond this limit the radius of the hologram is not increased further and keeps the maximum radius of \( R_o \). That is again because the size of the spherical wave projection on the detector exceeds the plane wave projection, so the overlap area remains within the same circle with the radius of \( R_o \).

To further investigate the properties of FINCH in comparison to a regular imaging system, one needs to equalize the size of both overall output images. Recall that the FINCH’s overall image of many points is bigger by the factor \( z_h / f_d > 1 \), hence the output image with FINCH should be shrunk by this factor. So, when the FINCH image is shrunk by the factor of \( z_h / f_d \), the overall image of both systems is the same and therefore can be compared on an equal
basis. However, the result of shrinking the entire image causes the PSF size of FINCH to be narrower by the factor of $z_h/f_d$ in comparison to that of a regular imaging system. Therefore, the effective width of the PSF of FINCH is

$$\Delta_x = \begin{cases} \frac{1.22\lambda z_h f_d}{R_o z_h} & f_d \geq \frac{1}{2} z_h \\ \frac{1.22\lambda z_r f_d}{R_o z_h} & 0 < f_d < \frac{1}{2} z_h \end{cases}$$

(10)

According to Eq. (10), the PSF width and consequently the resolution are dependent on the ratio $z_h/f_d$ for all values of $f_d$. Note that this dependence of the resolution to the ratio $z_h/f_d$ is different from the conclusion of Ref [21], where the authors there have claimed that above $z_h/f_d > 1$ the resolution is constant and is equal to that of a regular imaging system. The minimum width of the PSF is obtained for $z_h/f_d = 2$, and this width is $\Delta_x = 0.61 \cdot \lambda f_d / R_o$ (or $0.61 \cdot \lambda f_o / R_o$ in the object domain), which is half the width of the PSF of a regular imaging system [shown in Fig. 1(b)] with the same numerical aperture. The effective PSF of FINCH for the ratio $z_h/f_d = 2$ is now,

$$h_x(\tau) = C'' \cdot \text{Jinc} \left( \frac{4\pi R_o}{\lambda f_d} \sqrt{(x-M_r x_i/2)^2 + (y-M_r y_i/2)^2} \right).$$

(11)

<table>
<thead>
<tr>
<th>PSF</th>
<th>MTF</th>
<th>Type of Linear Operation</th>
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<tbody>
<tr>
<td>Coherent Imaging System</td>
<td></td>
<td>$A_x/h$</td>
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<td>$f_x$</td>
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<tr>
<td>Incoherent Imaging System</td>
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<td>$l_x</td>
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<td>$f_x$</td>
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<tr>
<td>FINCH (Hybrid imaging system)</td>
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<td>$l_x/h$</td>
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<td>$f_x$</td>
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Fig. 2. Summary of the main features of the three linear systems discussed in the text. $A_x$ and $l_x$ stand for a complex amplitude and intensity of the input object, respectively. $x$ and $f_x$ are the space and the spatial frequency coordinate, respectively.
In terms of resolution, the improvement of FINCH in comparison to a regular incoherent microscope is more than a factor of 1 but somewhat less than a factor of 2 because the PSF of FINCH shown in Eq. (11) has the shape of that of a coherent system. To estimate the resolution improvement we recall that according to the Rayleigh criterion, two points are resolved if the dip between their images is more than approximately 27% of the maximum intensity [11]. A simple numerical calculation indicates that in order to create a dip of not less that 27% between two functions of the form of Eq. (11), the minimal distance between them should be no less than $0.61 \cdot \delta_d / (1.4 \cdot R_o)$ and $0.61 \cdot \delta_d / (1.5 \cdot R_o)$ in cases of linear and non-linear reconstruction, respectively. Therefore the resolution improvement of FINCH over a regular incoherent microscope is about a factor of 1.4 and 1.5 for linear and non-linear reconstruction, respectively. The FINCH’s resolution improvement over a coherent imaging system is a factor of 2.

2.2. Discussion

According to Eq. (5), the PSF of FINCH is obtained as the scaled Fourier transform of the system aperture, exactly as is the case of a coherent imaging system. Therefore the shape of the MTF of FINCH is similar to the shape of the system aperture, i.e. a uniform clear disc shape. However the cut-off frequency of FINCH is different by the ratio of $z_h / f_d$ than that of a regular coherent imaging system, and can be twice as high in the optimal setup of $z_h / f_d = 2$. Moreover, FINCH with the ratio $z_h / f_d = 2$, has the same cut-off frequency as an incoherent imaging system, but unlike the later system, the MTF of FINCH is uniform over all the frequencies up to the cut-off frequency.

We conclude that FINCH is superior in terms of resolution over both coherent and incoherent imaging systems. In fact, FINCH enjoys the best of both worlds; it has a cut-off frequency of an incoherent system with the same numerical aperture, and a uniform MTF like a coherent system. Figure 2 summarizes the main properties of FINCH in comparison to either coherent or incoherent imaging systems. Looking at Fig. 2, one can conclude that, in addition to the two well known types of imaging systems, coherent and incoherent, there is a third type which can be denoted as a hybrid imaging system characterized by FINCH, since it associates incoherent recording with coherent reconstruction. The hybrid imaging system is linear in the intensity but its PSF is in general a complex valued function. Its MTF has the shape of the system aperture with a cut-off frequency that can be twice as large as that of a coherent imaging system with the same numerical aperture. In comparison to an incoherent system we see that both systems have the same bandwidth but FINCH does not attenuate the intensity of spatial frequencies greater than zero, as the incoherent imaging system does.

The superiority of FINCH in the resolution aspect is explained by the fact that the hologram of each object point is an interference result between two beams, both originated from the same point. The information about the point location is stored in the phase of both beams. During the wave interference, under the condition $z_h / f_d > 1$, the two phases have the same sign and therefore they are summed such that the resulting linear phase function has a higher slope than in case of recording a coherent hologram with a non-informative reference beam. Therefore, as a result of the phase gaining, the image point location is farther from some arbitrary reference point than in the case of a regular imaging system, and therefore the image magnification is higher in FINCH. As the result, the separation between points is larger in FINCH and this feature is translated to better overall resolution. In the regime of $z_h / f_d < 1$ the two summed phases have an opposite sign such that the resulting overall phase is demagnified, the gap between various image points, and consequently the resolution, are smaller in comparison to a conventional imaging system with the same numerical aperture.
In this study we compare the transverse resolution of two 2D imaging systems; the conventional incoherent imaging system and FINCH. For both systems we analyzed the resolution at the front focal plane of the objective in which a comparison could be made because conventional incoherent imaging only resolves a single plane of focus. While FINCH can resolve multiple planes in an image, an analysis of FINCH resolution was limited to the front focal plane in this study for comparison purposes. In the future, theoretical and experimental analysis of the resolution properties of a more general FINCH, in which the
location of the object is not limited to the front focal plane of the objective, should be very interesting and may offer additional opportunities for high resolution 3D imaging. Because FINCH utilizes an SLM it is possible to modify the diffractive lenses in the system and therefore to optimize the imaging resolution at different object planes.

All the above mentioned analysis is based on the assumption that FINCH is diffraction limited and the pixel size of the camera does not limit the system resolution. This assumption is fulfilled if the finest fringe of the hologram can be correctly sampled by the camera. Referring to Fig. 1(a) with the condition $z_h/f_d = 2$, and recalling that the finest fringe is created by the interfered beams with the largest angle difference between them, the condition that should be satisfied is

$$\tan \phi = \frac{2R_o}{z_h} \leq \frac{\lambda}{2\delta}, \quad (12)$$

where $\phi$ is the largest angle difference between the interfered beams in the system and $\delta$ is the camera pixel size. For a given SLM and digital camera, the only free variable is $z_h$. Therefore, in order to keep the system as diffraction limited as possible, the distance between the SLM and the camera should satisfy the condition, $z_h \geq 4R_o \delta / \lambda$. Increasing the distance $z_h$, while keeping the optimal condition $z_h/f_d = 2$, narrows the field of view. Based on geometrical considerations, the radius $R_o$ of the observed disk which can be recorded into the hologram is $R_o = 2f_0R_o/z_h$.

Based on the discussion above, it is clear that the optimal ratio in sense of resolution between $z_h$ and $f_d$ is $z_h/f_d = 2$. However this optimal ratio is obtained in the specific setup shown in Fig. 1(a) and the question is whether there is a more general configuration of FINCH in which the same resolution can be achieved. In the following subsection we try to answer this question.

### 2.3. Alternative FINCH configurations

According to Eq. (10) the effective resolution of FINCH is

$$\Delta_e = \frac{1.22\lambda z_r f_d}{R_H z_h}. \quad (13)$$

In order to improve resolution one should look for a configuration with higher $R_H$ and $z_h/f_d$ and with a $z_r$ that grows less than the other two factors. Such configuration might be the one shown in Fig. 3(a), in which the FINCH is generalized in the sense that the constant phase on the SLM is replaced with a negative lens with $f_2$ focal distance. When the various parameters are chosen such that there is a perfect overlap between the two spherical waves on the camera plane, $R_H$ and the ratio $z_h/f_d$ indeed become higher. The new $z_r$ is calculated from a similar equation to Eq. (1), in which in addition to the constant $B$ there is a transfer function of a negative lens as the following.

$$I_H(u,v) = \sqrt{I_s C(\tau)} L \left( \frac{-\tau}{f_o} \right) Q \left( \frac{1}{f_o} \right) Q \left( \frac{-1}{f_o} \right) Q \left( \frac{1}{d} \right) \times \left[ B Q \left( \frac{1}{f_2} \right) + B' \exp(i\vartheta) Q \left( \frac{-1}{f_d} \right) Q \left( \frac{1}{z_h} \right) P \left( R_H \right) \right]^2. \quad (14)$$

$z_r$ calculated from Eq. (14) is
\[ z_r = \frac{1}{2} \left( \frac{z_h - f_d}{f_d} \right) \left( \frac{z_h + f_d}{f_d} \right). \tag{15} \]

The transverse magnification remains \( M_T = z_d/f_o \) as before. Next, we make use of the fact that the two spherical waves perfectly overlap on the camera plane, and based on simple geometrical considerations, the following two relations are obtained,

\[ R_H = R_o \frac{z_h - f_d}{f_d}, \tag{16} \]
\[ \frac{z_h - f_d}{f_d} = \frac{z_h + f_d}{f_d}. \tag{17} \]

Substituting Eqs. (15)-(17) into Eq. (13) yields that effective width of FINCH’s PSF in the general configuration is

\[ \Delta_e = \frac{0.61 \lambda f_d}{R_o}. \tag{18} \]

This is the same result obtained with the configuration of Fig. 1(a) for \( z_h/f_d = 2 \). The conclusions are the following: 1) FINCH resolution in any configuration is limited by the value of \( \Delta_e \) given in Eq. (18). This conclusion is expected since any configuration of FINCH does not enable any new information, or more spatial frequencies, to enter into the system, and therefore there is no reason for any further resolution improvement beyond the superior result given in Eq. (18). 2) The optimal configuration can be obtained in many forms as long as the overlap between the two different beams on the camera plane is perfect. This conclusion is true even if both diffractive lenses on the SLM are positive, where one is focused before the camera and the other beyond it, as is shown in Fig. 3(b). In that case the \( z_r \) is calculated by the same method to be

\[ z_r = \frac{1}{2} \left( \frac{z_h - f_d}{f_d} \right) \left( \frac{z_h + f_d}{f_d} \right), \tag{19} \]

and the radius of the hologram under the perfect overlap condition is the same as is given in Eq. (16), where the following relation also exists:

\[ \frac{f_2 - z_h}{f_2} = \frac{z_h - f_d}{f_d}. \tag{20} \]

Substituting Eqs. (16), (19), (20) into Eq. (13) yields again the same effective resolution as is given in Eq. (18). Here again the optimal resolution can be achieved. The possible advantages of each configuration of Figs. 1(a), 3(a) and 3(b) should be investigated in the future. Note that displaying two different diffractive lenses on randomly distributed pixels of the same SLM could result in reduced efficiency from both lenses, because only half of the SLM pixels are available for each lens [14,15]. Therefore a glass spherical lens should be added to the system which together with the SLM (on which the pattern of a sum of constant and quadratic phase functions are displayed) creates an equivalent system of Fig. 3(b). This system is depicted in Fig. 3(c). The purpose of the additional glass lens is to convert the plane wave, reflected from the SLM, into a converging spherical wave which interferes with the other spherical wave in order to create the hologram.
3. Experimental methods

The purpose of these experiments was to test the theoretical predictions. Specifically, we wanted to determine the relationship between $z_n/f_a$ and FINCH resolution and to compare the resolution of FINCH microscopy at optimal $z_n/f_a$ to that of optical microscopy. Implementing FINCH holography in a microscope (FINCHSCOPE) only requires that the fluorescence microscope be changed in the way fluorescence emission is detected. The infinity beam of the sample imaged with a microscope objective is directed to an SLM and is split into two beams which interfere at a camera to create a hologram [10]. The microscope configuration schematically shown in Fig. 4 used for these experiments was built upon our laboratory’s previous concepts and designs for implementing FINCH in a microscope [15,16] with some important additions and modifications. In the experiments presented here, the identical smallest features on the highest resolution USAF chart were imaged at the plane of focus by three methods and compared; 1) conventional high resolution fluorescence microscopy with all glass optics including a matched and properly configured microscope tube lens, 2) microscopy which utilized the SLM as a tube lens to focus the image upon the camera and 3) holograms captured with FINCH and reconstructed at the best plane of focus.

In order to simplify analysis and be able to compare image resolution between conventional fluorescence microscopy (which only resolves a single focal plane) and FINCH, a USAF negative test slide (Max Levy Autograph) with a single plane of focus that contained group 9 features as small as 645 lp/mm (0.78 µm feature size) was used and was much smaller than the smallest features used previously [16]. The slide was placed upon a fluorescent plastic slide (Chroma), as we previously described [16], so that the negative features were fluorescent. A No. 1 coverslip was placed on the slide with microscope immersion oil between the coverslip and the test slide. There was an air interface between the objective and the top of the coverslip. The USAF pattern was adjusted to the plane of focus of the objective and kept in that position for all of the imaging experiments.

An important difference in the configuration from previous designs is that the SLM was positioned at a 45° angle and the system was designed for ready switching between ocular or camera viewing of the sample fluorescence and holography without disturbing the position or focus of the sample. This new microscope configuration was constructed on the stand of an upright Zeiss Axiophot fluorescence microscope. The binocular head with camera port and tube lens of the microscope was removed and the components needed for FINCH holography and viewing of the sample were attached to the microscope in its place. The remaining components of the microscope were not altered. An AttoArc 100 watt mercury arc lamp was used as the excitation source and the excitation was controlled by an electronic shutter. In these experiments, an air Nikon Plan Apo 20X, 0.75 NA objective was used. The epifluorescence dichroic and excitation filter were Semrock Cy3 filters, and the emission filters were a 570 nm center $\lambda$, 10 nm bandpass filter (Thorlabs) for the FINCH images and the images taken with the SLM as a tube lens. A Semrock Cy3 emission filter was used for the glass tube lens ocular viewing and camera images. In experiments not shown, as expected, the resolving power of the objective-tube lens combination was confirmed to be the same with the Cy3 emission filter as with the 10 nm bandpass filter. This is because the Nikon Plan Apo objective – tube lens combination is achromatic. A major improvement in light transmission was achieved by placing the SLM at a 45° angle and eliminating the beam splitting cube used in previous work [16]. Careful alignment of the SLM (Holoeye HEO 1080P, 1080x1920 pixels, 8 um pixel pitch, phase only) in all directions was essential to prevent any image degradation. Furthermore the SLM firmware was modified to give a $2\pi$ phase shift over its range at a 45° angle and the Fresnel patterns displayed on the SLM were adjusted for the 45° angle. Input and output polarizers were rotated 45° along the optical axis as previously described [16] for improved resolution, so that all the pixels on the SLM were utilized to create the two interfering wavefronts. As previously described [16], the 8 meter physical...
curvature of the SLM substrate was accounted for in the lens parameters used to generate the desired focal lengths created by the diffractive Fresnel lens patterns that were displayed on the SLM. A multi-axis micrometer controlled mount was constructed so that the SLM could be adjusted to be precisely centered on the optical axis and so that there was no rotational misalignment of the SLM about the optical axis. A calibrated iris was attached to the back aperture of the objective so that the back aperture could be varied from 3 mm to 12 mm to reduce the resolution of the objective so that FINCH imaging could be directly compared to optical microscopy at different effective objective NAs. Removal of the iris enabled imaging with the full 16 mm back aperture of the objective. In order to compare imaging performance between regular microscopy with that of FINCH, the microscope was configured so that a precision mirror on a roller-ball bearing slider could be inserted into the emission beam path without disturbing the location or focus of the sample or the setting of the back of the objective. Once the mirror was in place, the emission light was simultaneously directed through a Nikon tube lens and beam splitting cube to another of the same model camera that was used for holography. Furthermore, an ocular positioned on the beam splitting cube allowed direct viewing of the sample under observation. Both the ocular and both cameras were aligned and positioned to be precisely parfocal (all at the same focus) under imaging conditions at the correct focus position between the objective and sample. An in focus image on the camera used for holography was obtained when the focal length of the diffractive lens pattern displayed on the SLM was equivalent to the distance between the SLM and camera.

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**Fig. 4.** Schematic representation of the microscope for comparison of FINCH to standard fluorescence microscopy on the same identical sample without change in position or focus. The position of the two sliders and the diffractive lens pattern displayed on the SLM determine the imaging mode selected. The position of the sliders is shown for FINCH. Imaging of the sample using the SLM as a tube lens was possible by moving the input polarizer to the open position and displaying a diffractive lens pattern with a focal length equivalent to the distance between the SLM and camera. Reversing the position of the two sliders shown in the schematic allowed direction of the fluorescent emission to pass through the Nikon tube lens to the monocular viewing port and associated imaging camera for conventional fluorescence microscopy.
The two CCD cameras were QImaging Retiga 4000R, cooled 2048x2048 pixel, 7.4 µm pixel pitch, 12 bit.

The operation of the microscope was controlled by software written in LabView. Three phase shifted holograms were taken for each FINCH image and calculated as previously described [16].

4. Experimental results

The ability of the camera to resolve the fine fringes of the hologram has a significant effect on the ability of FINCH to resolve small objects. Because of this, we moved the camera away from the SLM until we reached a $z_h$ position of 1380 mm at which we were able to resolve the smallest features in the USAF pattern using FINCH with $\frac{z_h}{f_d} = 2$. The size of the acquired hologram is equal to the size of the diffractive Fresnel lens displayed on the SLM. As shown in the left panel of Fig. 5, the microscope image of the small features in groups 8 and 9 (shown in the red box) under standard imaging conditions with a tube lens and with a 5 mm aperture over the back of the objective lens, was quite small and needed to be zoomed in to see them as shown in the middle image of Fig. 5, while the FINCH images needed to be zoomed and cropped much less due to the magnification imposed by the long SLM-CCD distance. As can be seen, the small features were not well resolved by regular microscopy, however imaging with FINCH clearly resolved the small features as shown in the right panel of Fig. 5.

![Fig. 5. Representative full field USAF slide images captured in standard microscope operating mode (left panel). Middle panel: zoomed-in group 8 and 9 features from full field standard microscope image. Right panel: Digitally linear reconstructed FINCH image of the small central pattern shown in the middle image, slightly cropped to match the middle image. All images were taken with a 5 mm aperture placed at the back plane of the objective.](image)

The USAF resolution target used in these experiments contains the smallest features available. In order to compare FINCH resolution in a very controlled manner to standard microscopic imaging, we imaged this target with the Nikon 20X 0.75 NA objective which had a 16 mm back aperture. We then installed a calibrated iris (Thorlabs) on the back aperture of the objective and systematically reduced the aperture from 12 mm to 3 mm. At each reduction in the back aperture, we took standard microscope images, images using the SLM as the tube lens and FINCH holographic images which were reconstructed as either linear or non-linear images as described above. Results from using 3, 5, 8 and 16 mm (no iris) back apertures are shown in Fig. 6.
Fig. 6. Cropped sections of images taken with: standard Nikon tube lens configured for standard fluorescence microscopy (first column); with the SLM acting as a tube lens (second column); and with either the linear and non-linear reconstruction of FINCH holograms. The FINCH images were recorded with a z-ratio of 1.8. Images with the SLM as the tube lens or with the FINCH method were taken at a SLM-camera distance of 1380 mm. The four sets of images were taken with varying apertures in the back plane of the objective as indicated on each row.

Additional apertures of 4, 6, 10 and 12 mm were used with results intermediate to the images shown here. An analysis of this experiment is shown in Fig. 7. The plot of Fig. 7 shows the visibility in the smallest group of lines versus the diameter of the back aperture, where the visibility defined as \( \frac{I_{\text{max}} - I_{\text{min}}}{I_{\text{max}} + I_{\text{min}}} \) is a standard quantity used to characterize resolution. In this work, we examined visibility of the horizontal features in group 9, element 3, i.e. the smallest features. To define \( I_{\text{max}} \), we located the row of pixels in each of the three features that had the highest summed intensity. We then averaged all the pixel values from those rows. To define \( I_{\text{min}} \), we located the row of pixels in each of the gaps between the features that had the lowest summed intensity, and then averaged the pixel values from those rows. Visual inspection of the images and the visibility calculations demonstrate that FINCH images resolve the smallest features better than images from the comparable standard microscope configuration at all effective NAs of the objective. Using the SLM as a tube lens produced images which had similar resolution to the glass tube lens up to an aperture of 8 mm, the approximate minimum size of the aperture of the SLM when viewed at a 45° angle in our setup.
We then investigated the relationship between resolution and $z/f_d$, which we call $z$-ratio, using a reduced aperture of 5 mm since this dramatically reduced the imaging resolution of the objective under normal microscope conditions. Images at varying $z$-ratios from 0.85 to 2.4 were recorded and are shown in Figs. 8 and 9. Visual inspection of the images shows that the resolution continues to improve as $z$-ratio increases from 0.85 and reaches a peak around $z$-ratio $= 1.8 \pm 0.2$. Visibility data is presented in Fig. 10. The maximum is not exactly at $z_h/f_d = 2$ because as already indicated in Ref [16], the SLM has inherent spherical-like curvature which introduces an effective positive spherical lens of about 8 meter focal length. In other words, instead of a system of the type shown in Fig. 1(a) in which the maximum resolution is obtained at $z_h/f_d = 2$, effectively there is a system of the type shown in Fig. 3(c) in which there is an additional lens in the system (the inherent 8 meter curvature of the SLM) and the maximum resolution is obtained at about $z_h/f_d = 1.8$. Note that although the focal length of the diffractive lens displayed on the SLM is corrected to account for the inherent curvature of the SLM, the constant phase mask cannot be corrected, and therefore the model shown in Fig. 3(c) is valid here. This system behavior is in contrast to the report by other investigators [21] that there was no change in resolution between $z$-ratio of 1 and 2. Note that at $z$-ratio $= 0.85$ the visibility in the smallest group of lines is zero and therefore this point of data is not included in the plot of Fig. 10. However this result fits the prediction that the resolution of FINCH for $z$-ratio<1 is lower than that of a regular microscope; as seen in Fig. 7, the visibility of the smallest group of lines, with objective back aperture of 5 mm, is 0.1.
Fig. 8. Linear reconstructions of FINCH images taken at varying z-ratios. At low z-ratio below 1, the SLM is focusing behind the camera while at high z-ratio above 1, it is focusing in front of the camera. Images were taken with a 5 mm aperture at the back plane of the objective, with a z_0 of 1380 mm.
Fig. 9. Non-linear reconstructions of FINCH images taken at varying z-ratio values. At low z-ratio below 1, the SLM is focusing behind the camera while at high z-ratio above 1, it is focusing in front of the camera. Images were taken with a 5 mm aperture at the back plane of the objective, with a z-h of 1380 mm.
Fig. 10. Plots of the visibility of the three smallest USAF features in FINCH as a function of the $z$-ratio, taken with a 5 mm aperture in the back plane of the objective. Data for both linear and non-linear reconstructions are shown. These data were taken with a $z_h$ of 1380 mm. For comparison, the visibility in standard microscopy is approximately 0.1 when the aperture is 5 mm (see Fig. 7). The lines are a polynomial fit of the data. For FINCH Non-linear, $y = -0.5769x^2 + 2.1313x - 1.1801$ $R^2 = 0.8074$ and for FINCH Linear, $y = -0.4848x^2 + 1.7946x - 1.1604$ $R^2 = 0.7866$.

5. Conclusions

We have analyzed the FINCH with the tools of the linear system theory. The theoretical conclusions are supported well by experiments described herein. The main conclusions are:

1. FINCH is a hybrid system in the sense that its MTF has the shape of a coherent imaging system but in the optimal conditions, its spatial bandwidth is equal to that of an incoherent system.

2. The width of the PSF of FINCH, and accordingly its resolution, is dependent on its configuration and on the ratio between the distance from the SLM to the camera and the focal length of the diffractive lens. In all the possible configurations, the condition to obtain maximum resolution occurs when there is a perfect overlap between the projections of the two different interfering beams (originating from the same point source) on the camera sensing plane.

3. Under the optimal condition described in item 2, FINCH can resolve better than a regular glass-lenses-based imaging system with the same numerical aperture. In terms of Rayleigh criterion the improvement is between 1.5 and 2 fold in comparison to incoherent and coherent systems, respectively.

The experimental data very well supports our theoretical predictions. First, we have shown that indeed, the resolution of FINCH at the focal plane is better than that of a regular microscope with the same numerical aperture. The native microscope objective yielded better resolution with FINCH than with standard imaging. Furthermore, reduction in the back
aperture over a wide range shown in Fig. 7 enabled us to demonstrate significantly greater resolution with FINCH compared to standard microscope imaging. Moreover, as the aperture size decreased, the graph of the visibility drops much more steeply in the case of FINCH than in the case of the glass tube lens, indicating that its MTF is more uniform in the range below the cut-off frequency. Because FINCH resolution at the focal plane exceeds standard imaging methods, a natural outcome of our present experiments will be to extend the theoretical analysis and experimental verification to 3D objects which by standard imaging methods are out of focus above and below the focal plane but are resolved by FINCH.

In the second experiment we verify the relationship between resolution and the ratio \( z_h/f_a \). As predicted theoretically, the curve of visibility versus \( z_h/f_a \) is not flat [21] but has a maximum value not far from the predicted ratio \( z_h/f_a = 2 \).

Although all of the experiments in this study refer to a fluorescence microscope it should be emphasized that FINCH can be applied to any incoherently illuminated microscope and even to any incoherent imaging system. The theoretical and experimental data presented here makes FINCH an attractive platform for a very simple super-resolution system that can resolve better than any conventional imaging system with the same numerical aperture.

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