Investigating the applicability of LCD technology to structured illumination microscopy

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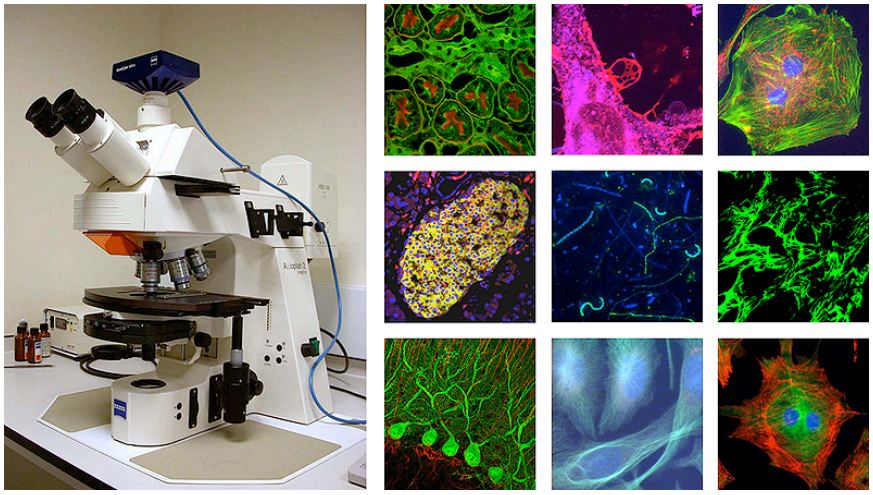
Fluorescence microscopy



- Fluorescence microscopy conquers the world
- Non-destructive
- Allows viewing of live samples in physiological conditions
- That's what biology is all about

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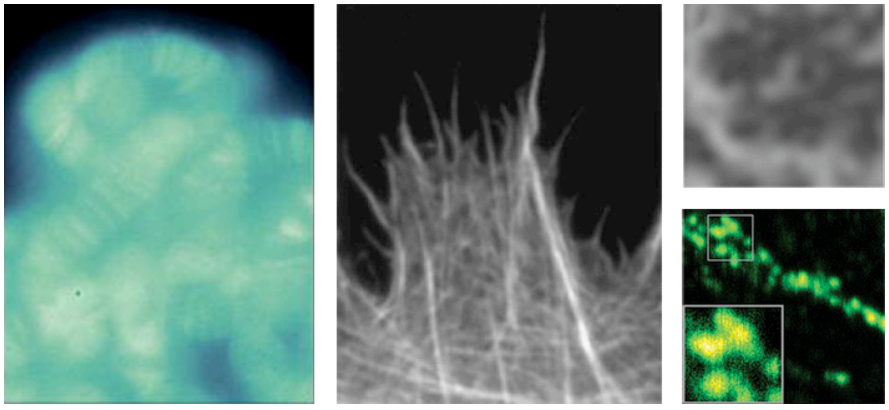
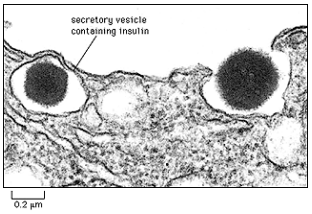
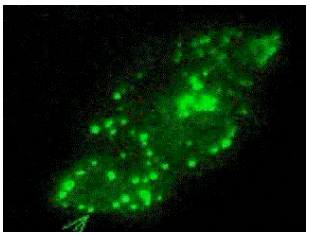
A hard constraint

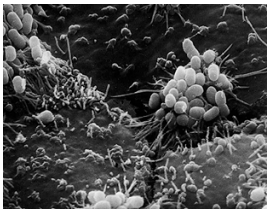


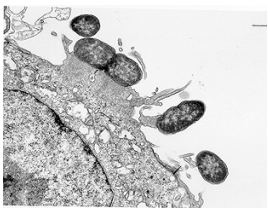
- As you look at smaller things, diffraction comes into play
- Things fuzz out
- It's difficult to see what's going on
- Which is a shame, because there's plenty of interesting stuff going on down there

So near and yet so far









•Obviously, that's always true -- there's no end to potentially interesting detail

•But there are a plenty of important processes that are only *just* out of reach

•Dense core granules package and release hormones from neuroendocrine cells

•Microvilli increase surface area, seen here being destroyed by enteropathogenic e coli -- this makes you ill!

•There ARE other methods for imaging this -- as seen here, plus scanning probes, etc -- but mostly destructive, heavyweight, surface-bound etc

•Ideally, we'd like to push the advantages of ordinary fluoro microscopy into this more detailed range.

•Structured illumination is one way to do this; to see how it works, we need to think in terms of frequency domain.

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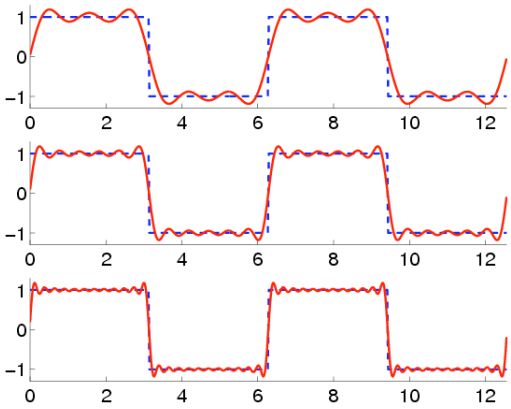
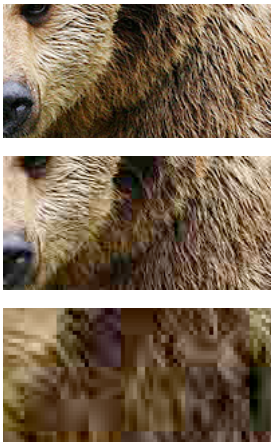
Detail is high frequency information





- As the title says, small details correspond to high frequencies
- Without those frequencies, things tend to be pretty wobbly even if the vague shape is there
- Eg, the classic Fourier picture of a square wave built from harmonics (left) or JPEG compression (right)

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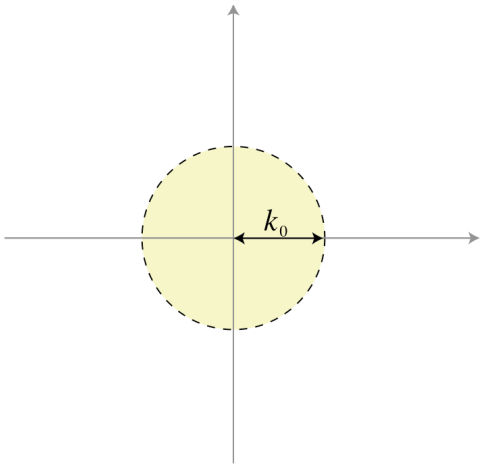
The diffraction limit is a frequency cutoff



$$k_0 = \frac{2\,NA}{\lambda}$$

- In these terms, diffraction limit defines a maximum observable region of the frequency domain
- Based on wavelength and numerical aperture
- (back of envelope calc, lambda = 600nm, NA = 1.5, k0 approx 5 MHz)
- For better resolution, need to smuggle details from outside this region back into it
- That's what structured illumination does, by exploiting interference fringes between sample and a pattern in the incident light

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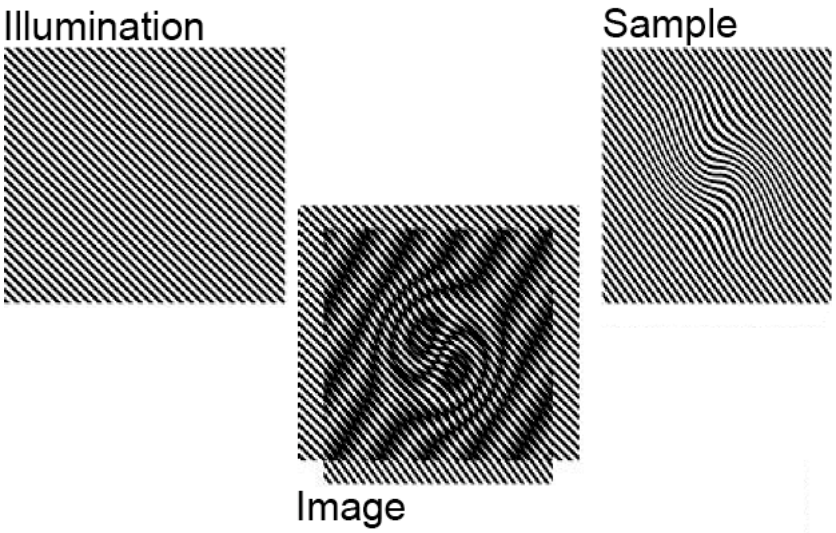
Moiré patterns



•Illumination is patterned
•Sample contains high frequency information
•Moiré fringes are low frequency, safely within the observable region
•Higher resolution image can be recovered by using multiple images
•Imposed pattern ALSO bound by diffraction limit
•Double resolution with linear techniques, more with non-linear
•BUT, requires increasing numbers of images, which brings us to…

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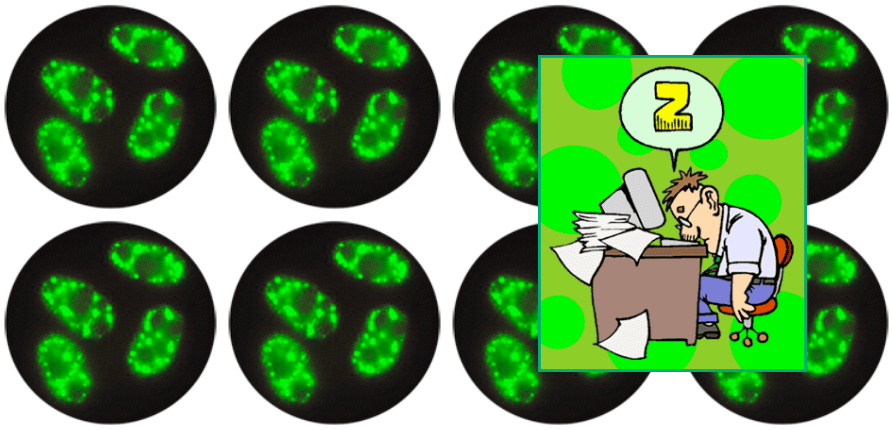
The rub



- Method works, but image capture is slow
- Low time resolution would be bad enough, but worse is that whole method depends on immobility across frames
- Thus, slow capture can't deal with moving samples at all
- Bummer for biology :(
- This is the point of this project

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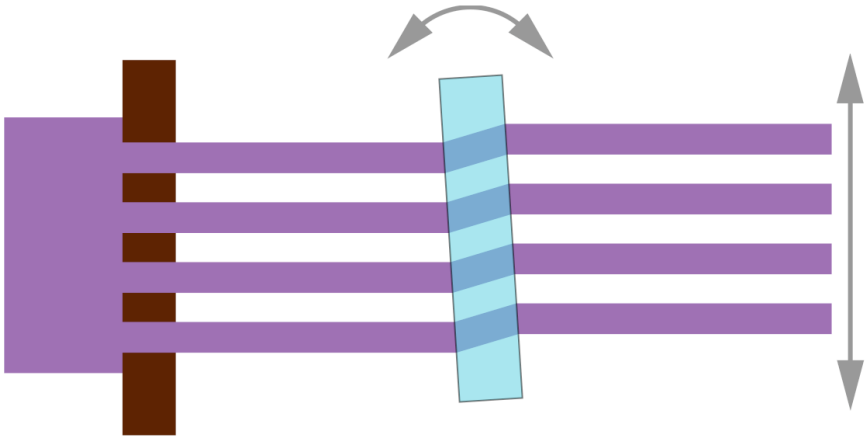
Repositioning the pattern



- Major sources of delay:
  - Physically moving the glass element
  - Synchronizing with frame capture
- Note this method also limits to one dimension

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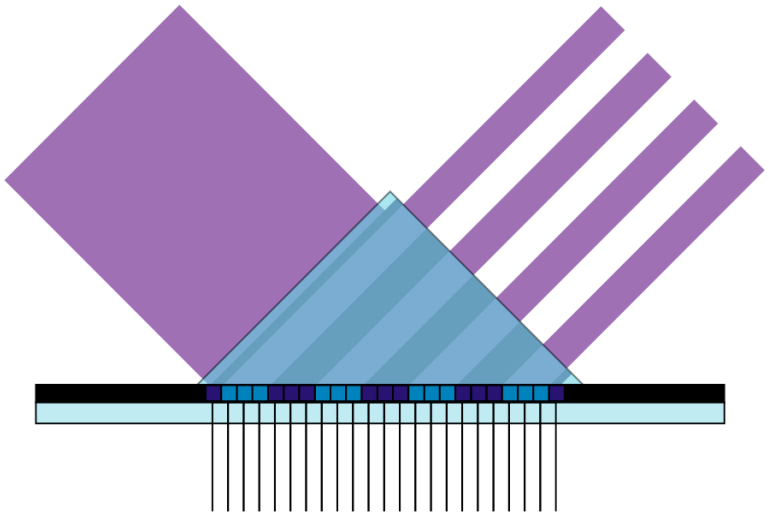
Replace with LCOS component?



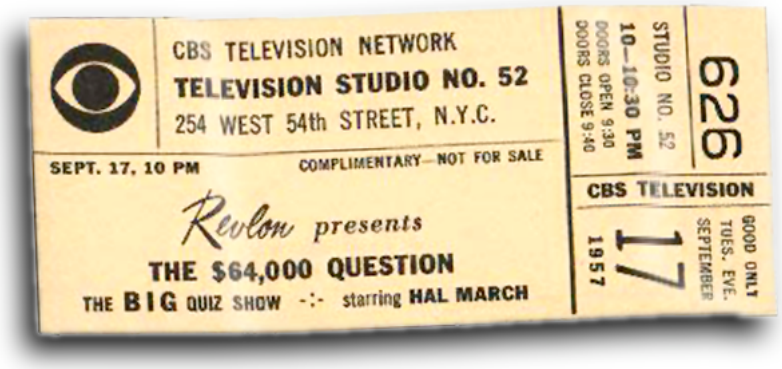
•Project: can we substitute a liquid crystal device under computer control

•On face of it should be faster and more versatile, allowing interesting new patterns, etc

•Potential problems:

- •Optical properties of LCOS, can we generate clean enough pattern to recover meaningful data
- •Issues of angles lines on rectangular pixel grid, introducing high freq edge components
- •etc

So: will it work?



•Well, if we knew that there wouldn't be a project.

•Come back in 3 months and find out!

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Questions?

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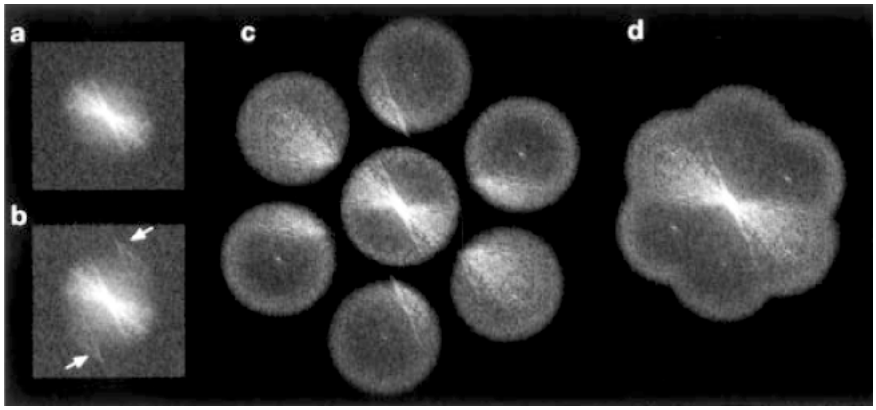
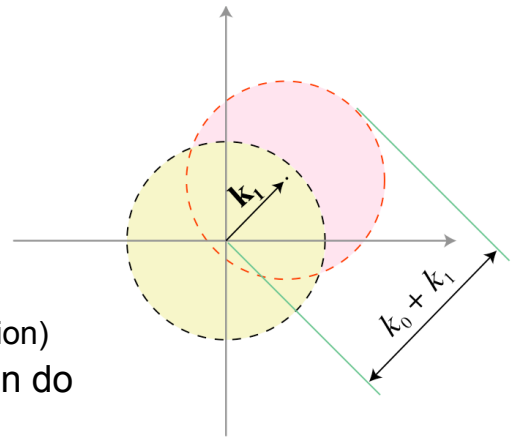
Reconstructing the image



How much of an improvement is it?

- Fringes visible if
  $|\mathbf{k} - \mathbf{k_1}| < k_0$
- $\mathbf{k_1}$ diffraction limited
- In linear case,
  $k_{max} \approx 2k_0$
  (ie, ~doubled resolution)
- Non-linear options can do better, **but…**



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Other imaging techniques?