ARI-RR-449

TOWARD AUTOMATED ANALYSIS OF PARTICLE HOLOGRAMS

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January 1985

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ABSTRACT

A preliminary study of approaches for extracting and analyzing data from particle holograms concludes that

- For "thin" spherical particles out-of-focus methods are optimum,
- For "thin" nonspherical particles out-of-focus methods are useful but must be supplemented by in-focus methods, and
- A complex method of projection and back projection can remove the unwanted out-of-focus data for "deep" particles.

I. INTRODUCTION

Particle holograms are capable of instantaneous recording of a large three-dimensional (3D) field particles. Given a particle hologram, we might have two tasks:

1. Find all of the particles and
2. Characterize them.

The verbs "find" and "characterize" must be defined more carefully for each particular situation. In most cases this analysis is carried out using a human observer to

1. Locate the plane of best focus for each particle,
2. Record the in focus image,
3. Characterize that image.

There are two major difficulties with this human-operator-based analysis: one obvious and one slightly more subtle. The obvious difficulty is that human-based analysis is notoriously slow, nonrepeatable, and (hence) costly and inaccurate. It is this analysis bottleneck which keeps particle holography equipment on the shelf while far less powerful technologies perform a few of the tasks holography could perform. The less obvious difficulty is that human-based analysis may be inherently less accurate than analysis based on a computer oriented algorithm.

This paper explores past and current approaches to computer analysis of fields produced by particle holograms based on "multiplane" algorithms. Multiplane algorithms are based on data obtained in several depth planes (rather than simply the "focal" plane).

II. PHILOSOPHICAL BACKGROUND

A human observer looking at a point of laser light will see that point clearly. A recording surface moving through the field produced by such a point will produce a point image in focus and a diffraction pattern out of focus. This assymetry between the human (who sees only what is in focus) and the camera (which sees the out of focus data as well) leads to different data analysis schemes. In principle, all of the information in the particle wavefront is contained in every plane (including, of course, the hologram plane). Nonholographic cameras do not record the whole information, however, so observations in different planes give different information.

To these fundamental observations we must add some practical ones. Particle hologram wavefronts are noisy and complicated. Other particles are present. Refractive artifacts occur. These complications mean that otherwise-equivalent observations are not really equivalent. More observations can mean reduced influence of these "noise" effects.

III. ANALYSIS OF SPHERICAL PARTICLES

Spherical particles are guaranteed in some cases, e.g. bubbles. For known spheres only two questions can be asked:

1. Where are they?

and

2. What are their diameters?

The old (human-oriented) approach is to move to the focal plane and there measure the lateral (x-y) position of the particle's center and measure its diameter. The depth (z) dimension is that of the camera in the focal position.

In the newer (computer oriented) approach, all of these quantities are measured in out of focus planes. We will review this past work briefly here.

The first work in this field was by Vikram and Billet.[1] They showed that diameter determination was far more accurate out of focus than in focus simply because the pattern is, in effect, magnified. Two derivative observations can be made immediately:

1. Localized film or detector noise is less of a problem out of focus than in focus and

2. The centroid of the sphere (x-y location) can be located more accurately out of focus than in focus.

The second work in this area was by Stanton, et.al.[2] They showed that

1. The depth of the particle could be found more accurately by out of focus measurements than by in focus measurements and

2. This obviates the need for searching all depth planes.

The explanation of the increased accuracy in depth (z) location is a fairly universal one worthy of a little more explanation. If we plot "spot" size, s, versus z ($z \equiv 0$ in focus), we obtain a curve with a minimum at $z = 0$. We can seek to find $z = 0$ by the z finding minimum s. Or we can measure s at two $z \neq 0$ positions on the same side of $z = 0$ and extrapolate to $s = 0$. The z sensitivity

$$\alpha = \left|ds/dz\right|$$

is a maximum away from $z = 0$ and minimum at $z = 0$. Indeed maximum α occurs at maximum numerical aperture and way from $z = 0$.

IV. SHALLOW OBJECTS

A shallow object is one with unresolvable depth information. All that counts is its two-dimensional cross section normal in x-y. The questions which can be asked include

1. What is the x-y-z centroid location?

2. What is the shape? (Not a well defined question) and

3. What is the orientation?

Whether these questions can be answered out of focus depends on what we mean by "shape" and "orientation." Suppose we mean by "shape" the best fit enclosing rectangle and by "orientation," the direction of the rectangle's predominant direction. Then clearly these are accessible out of focus. On the other hand many details of shape ~~or~~ are simply not detectable out of focus, e.g. a particle shaped like a "6" and a particle shaped like a "9".

When details of particle shape are of interest, we must go to a focal plane. This does not mean, however, we should ignore the out of focus information. Extrapolation from out of focus data is still probably the best way to obtain focus. Furthermore, the out of focus data can be used, at the price of considerable computational complexity, to improve our knowledge of the in focus image. The idea, of course, is to use some generalized Gerchberg[3] algorithm to iterate back and forth between the in-focus image and the out-of-focus diffraction pattern using

- Known Fraunhofer diffraction laws,
- Measured data in both domains, and
- Imposed constraints in both domains (e.g. nonnegativity in the image plane).

Such techniques are exceedingly powerful[4] and would, no doubt, be useful here as well.

V. DEEP OBJECTS

The hardest problems for any automatic, semiautomatic, or even human analysis is the deep object. Such an object is deeper than the depth of focus of its hologram. It is never all in focus in any plane. Of such objects we ask the usual questions (shape, size, 3D orientation, 3D location), but finding the answers is quite difficult.

The first problem which must be solved is that of separating in-focus images from out of focus artifacts. In any depth plane there are probably some of both. We suggest here an automated approach not as a final solution but as a starting place for more sophisticated analyses.

We might begin by projection of the 3D scene into 2D. To do this we sort all x-y pixels by some focus criterion. That is at each x_i, y_i we examine each discrete depth. We might estimate focus by brightness. Let the intensity at x_i, y_i in the k^{th}/ depth slice be I_{ijk}. We define

$$P_{ijk} = \max \begin{cases} I_{ijk} \\ P_{ij(k-1)} \end{cases}$$

That is, P_{ijk}, is the largest of the I_{ijk}'s seen so far. By the time we have sorted through all N depth slices, P_{ijN} is a "projection" of the 3D image into 2D (i,j). All in focus pixels regardless of their depth are collected in one plane.

The next step would be to reproject P_{ijN} back into 3D. The 3D reprojection of P_{ijN} is

$$R_{ijk} = \begin{cases} I_{ijk} \text{ if } I_{ijk} = P_{ijN} \\ 0 \text{ otherwise} \end{cases} .$$

Thus R_{ijk} should have no out of focus parts. The subsequent analysis must group non-zero R_{ijk} components into likely particles, characterize them, etc.

VI. PROSPECTS

The sole objective of this paper is to point out numerous opportunities to explore automated computer analysis of 3D particle fields obtained by holography. For shallow spheres the analysis is easy and only implementation is needed. For other shallow objects some analogous work can be borrowed from the spherical case but much new work is required. For deep objects, no work has been done. The critical observation, however, is that full automation appears to be within our grasp.

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