

Theory of the Apparatus and Theory of the Phenomena: The Case of Low Dose Electron Microscopy

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1. Introduction

Electron microscopy, and in particular low dose electron microscopy, offers interesting cases of experimental techniques where the theory of the phenomena studied and the theory of the apparatus used, are intertwined. A single primary exposure usually does not give an interpretable image, and computerized image enhancement techniques are used to create from multiple exposures a single, visually meaningful image. Some of the enhancement programs start from informed guesses at the structure of the specimen and use the primary exposures in a series of corrections to arrive at a image that can be read by trained observers.

In this paper I describe in the general deterministic case the possible relations between this phenomena theory and instrument theory. I give a Bayesian criterion for when an experiment is a test of the theory of the apparatus, rather than a test of the theory of the phenomena, and describe strategies used to ensure that tests of the theory of the phenomena are possible. I end by extending this framework to low dose electron microscopy which has a stochastic instrument theory and which provides an exception to a thesis by Robert Ackermann on the independence between theory and instrumentation. In low dose electron microscopic imaging of macromolecules, a change in theory may lead to a change in how the instrument behaves.

2. Theory of the Apparatus and Theory of the Phenomena

In testing theories the theoretical predictions e are often unobservable, and one needs instrumentation to produce observable events o that correlate, possibly after having been adjusted in various ways, with theoretical predictions. Thus, to evaluate a theory of the phenomena T in the light of observations one needs a theory of the apparatus A that interprets meter readings as values of physical quantities, provides adjustment factors to correct raw data, etc. Usually, experimental outcomes are directly reported in terms of values of theoretical quantities, together with a discussion of bounds for probable precision and accuracy, and the apparatus and whatever we need of its interpreting theory A is taken for granted, or "ready-at-hand". But the theory of the apparatus may itself be uncertain in ways that affect inference and a thorough discussion of the impact of o on T has to involve a discussion of A . In this paper I will consider

the low dose electron microscope, a measuring instrument in a wider sense. What makes any piece of equipment a measuring instrument is that a prescribed functional relationship between input and output is maintained (Finkelstein 1977). In other words, a measuring instrument should satisfy the *faithful measuring postulate*: there should be a stable, may be probabilistic, relationship between measurand e and signal o , and the measurand should be recoverable from a set of signals through inverse inference based on an information theoretic analysis of the instrument. That is, in the simplest case, the theory of the apparatus A should imply statements of the form ' $e \leftrightarrow o$ '. Thus, when we believe that a piece of equipment is a measuring instrument we are confident that such a functional relationship does exist, even though we may be uncertain about the precise nature of the relationship.

Questions that arise for an epistemology of scientific instruments are these. What inferences can be drawn from o regarding the theory of the phenomena and the theory of the apparatus? What are the possible relations between these two type of theory, and how do these effect inference? What reasons does one need for accepting a device as a measuring or observational instrument, that is, what are the grounds for accepting that the instrument satisfies the faithful measurement postulate? How do we determine that an experiment provides a test of the theory of an apparatus rather than of a theory of some phenomenon? Our intuition is that an instrument whose theory is ill understood cannot deliver convincing evidence, neither in favor nor against a theory; can this intuition be systematically backed in a theory of confirmation?

In this paper I will use the framework of Bayesian probability theory and of inverse reasoning through Bayes's theorem to start sorting out some of these questions. I take the probabilities involved to be 'quasi-objective'. The numbers involved should not just reflect the personal opinions of an individual scientist, but have to be backed by convincing arguments, or constructed through an acceptable design that is reasonable in the context of application (Shafer 1981; Shafer and Tversky 1985). I believe that an information-theoretic analysis of instruments is an especially rich source of these 'quasi-objective' probabilities, since they can be derived from the information flow chart of the instrument. However, in this paper I can only hint at this when I discuss the low dose electron microscope.

3. A Bayesian Analysis

Jon Dorling has given a Bayesian analysis of the Duhem-Quine problem: a scientific theory T generally does not imply testable predictions on its own, but only in conjunction with additional hypotheses H . Let me call this the double theory problem. If a prediction is shown false, how is one to decide where to put the blame: on T , on H , or on both? Dorling has shown how the constraints of coherency and conditioning forces a Bayesian to distribute, in the case of a faulty prediction made on the basis of a theory T and additional hypotheses H , blame over theory and additional hypotheses (Dorling 1979). For a number of historical cases he has argued that the scientists involved had prior probabilities P such that the bulk of the negative impact of the faulty prediction was carried by the additional hypotheses H . Thus $P(H|\bar{e}) \ll P(H)$ while $P(T|\bar{e})$ was only moderately smaller than $P(T)$. From the perspective taken in this paper, however, the question arises whether the experiments involved were not more tests of the theory of the apparatus rather than tests of the phenomena theory.

The relation between theory of the apparatus A and theory of the phenomena T is structurally similar to the double theory problem. Let T be a theory incorporating enough additional assumptions to make predictions e possible. Thus T implies e , and $P(e|T) = 1$. But e is unobservable, and one needs instrumentation to produce observ-

able events o that correlate, possibly after having been adjusted in various ways, with theoretical predictions. A theory of the apparatus A is such that $P(o \leftrightarrow o|A) = 1$. Thus $P(o|TA) = 1$. Either o or $\sim o$ is observed.

In a Bayesian analysis, the information that o is the case will be incorporated through conditioning, that is, through the conditional measure $P(-|o) = P(- \& o)/P(o)$. Thus the impact of o on T is a function of the prior probability $P(T)$, and the posterior probability $P(T|o)$.

By Bayes's Theorem

$$P(T|o) = \frac{P(o|T).P(T)}{P(o|T).P(T) + P(o|\sim T).P(\sim T)}$$

which shows immediately an important feature of the Bayesian approach: the impact of o on T is a function of the probability of the observation o assuming other positive-probable alternatives to T . Thus, a Bayesian analysis forces one to think of an exhaustive partition $\mathbb{T} = \langle T_1, \dots, T_n \rangle$ of possible theories that may account for the phenomena under study. Similarly there is a partition $\mathbb{A} = \langle A_1, \dots, A_n \rangle$ of possible instrument theories. Each cell from the grid $\mathbb{T} \times \mathbb{A}$ is, in the deterministic case, a theory pair $T_i A_j$ that makes a prediction o_{ij} with probability 1. I call these model-dependent probabilities. Thus, for the remainder of this paper, we have to realize that expressions as $P(o|T \& \sim A)$ are mixtures of subjective probabilities and model-dependent probabilities. In particular

$$P(o|T \& \sim A) = \sum_{i=1}^m P(o|T \& A_i) P(A_i | T \& \sim A)$$

There are a number of numerical relations we may consider to sort out the impact of observations on the theory of the phenomena and the theory of the apparatus. First, there are the ratios $P(T|o)/P(T)$, and $P(A|o)/P(A)$; or, in case $\sim o$ is observed, the ratios $P(T|\sim o)/P(T)$, and $P(A|\sim o)/P(A)$. In the case where a theory H implies an observation d , we know that $P(H|d) \geq P(H)$, and thus $P(H|d)/P(H) \geq 1$: d cannot be negative evidence for H . One of the surprises in double theory situations is that there are (logically possible but degenerated) cases where both $P(T|o)/P(T) < 1$ and $P(A|o)/P(A) < 1$ even though $P(o|TA) = 1$. I leave this as an exercise for the reader, and assume in the following discussion that this is ruled out.

For us, the interesting relation is between the ratios

$$(1) \frac{P(T|o)}{P(A|o)} \quad \text{and} \quad (2) \frac{P(T|\sim o)}{P(A|\sim o)}, \quad \text{in comparison to} \quad (3) \frac{P(T)}{P(A)},$$

since these will indicate the differential impact of observing o or $\sim o$ on A and on T . When (1) > (3) the confirmed prediction that o has had a relatively greater positive impact on T than on A .

Assuming that the theory of the apparatus is not formally inconsistent with T , we get

$$\frac{P(T|o)}{P(A|o)} = \frac{P(TA|o) + P(T\sim A|o)}{P(TA|o) + P(\sim TA|o)} = \frac{P(o|TA).P(TA) + P(o|T\sim A)P(T\sim A)}{P(o|TA).P(TA) + P(o|\sim TA)P(\sim TA)} = \frac{P(TA) + P(o|T\sim A)P(T\sim A)}{P(TA) + P(o|\sim TA)P(\sim TA)}$$

since $P(o|TA) = 1$.

Thus (1) > (3) iff

$$(4) \quad \frac{P(TA) + P(o|T\sim A)P(T\sim A)}{P(TA) + P(o|\sim TA)P(\sim TA)} > \frac{P(T)P(TA) + P(T\sim A)}{P(A)P(TA) + P(\sim TA)} =$$

The conditions under which (1) > (3) become somewhat more transparent if we assume that T and A are independent. In that case (4) reduces to

$$P(A) + P(o|T\sim A).P(\sim A) > P(T) + P(o|\sim TA)P(\sim T)$$

Thus by increasing $P(A)$, that is by obtaining other evidence o^* , irrelevant to T, such that $P'(A) = P(A|o^*) > P(A)$ we can guarantee that

$$\frac{P'(T|o)}{P'(A|o)} > \frac{P'(T)}{P'(A)}$$

This is illustrated in figure 1.

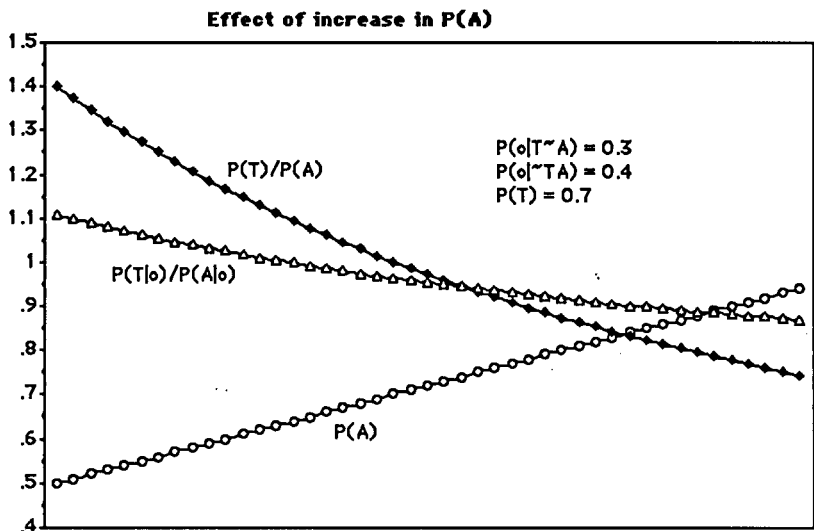


Figure 1

Furthermore, by increasing $P(o|T\sim A)$ we can make sure that the confirmed prediction that o has a relatively greater positive impact on T than on A . Thus, to focus on the most simple case where there is only two alternative theories of the apparatus in the partition $\mathcal{A} = \langle A, A_1, A_2 \rangle$,

$$P(o|T\&\sim A) = P(o|T\&A_1)P(A_1|T\&\sim A) + P(o|T\&A_2)P(A_2|T\&\sim A)$$

Now suppose that $P(o|T\&A_1) = 1$ and $P(o|T\&A_2) = 0$; we can increase $P(o|T\sim A)$ by collecting evidence o^* such that $P(A_2|T\&\sim A\&o^*) < P(A_2|T\&\sim A)$, and consequently $P(A_1|T\&\sim A\&o^*) > P(A_1|T\&\sim A)$. This is collecting evidence against alternative instrument theories that disagree on the prediction that can be made in the context of T . Figure 2 illustrates the effect.

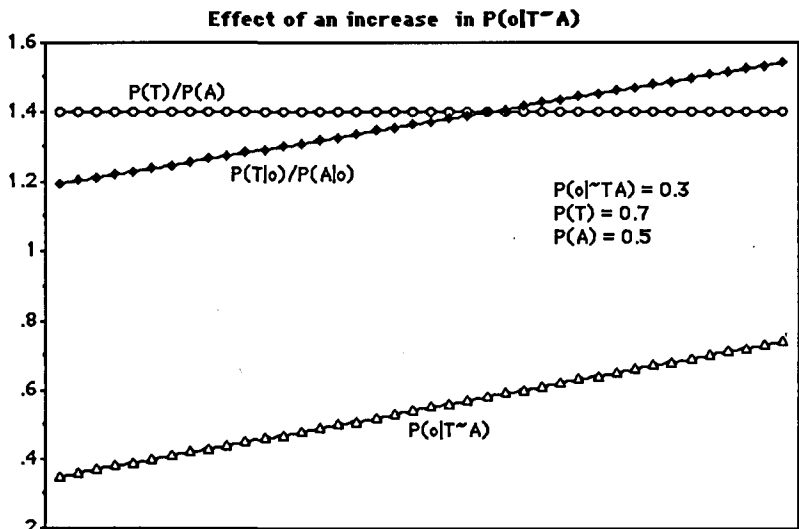


Figure 2

A similar effect is obtained by collecting other evidence that decreases $P(o|T\sim A)$. This is evidence that lowers the probability of alternatives to T that also imply o in the context of A .

We now consider the case of a faulty prediction. Thus, suppose again that $T\&A$ implies o , but that $\sim o$ is observed. The disconfirming impact of observing $\sim o$ is blamed mostly on T iff

$$\frac{P(T|\sim o)}{P(A|\sim o)} = \frac{P(\sim o|T\&\sim A).P(T\&\sim A)}{P(\sim o|\sim T\&A).P(\sim T\&A)} < \frac{P(T)}{P(A)}$$

i.e. iff

$$P(\sim o|T\&\sim A).P(T\&\sim A)P(A) < P(\sim o|\sim T\&A).P(\sim T\&A).P(T)$$

assuming independence this reduces to

$$P(\sim o|T\&\sim A).P(\sim A) < P(\sim o|\sim T\&A).P(\sim T).$$

Thus, the same strategies that are used to make sure that a successful prediction especially increases the posterior probability of the theory of the phenomena T [increase $P(A)$, or $P(o|T \& \sim A)$] also guarantee that an unsuccessful prediction will particularly hurt the theory of the phenomena.

4. Test of Phenomena Theory rather than of the Apparatus Theory

The discussion of the previous section suggests a somewhat different approach. Remember that a Bayesian analysis of the interaction between instrument theory and phenomena theory draws attention to the two partitions $\mathbb{T} = \langle T_1, \dots, T_m \rangle$ of phenomena theories of positive probability and $\mathbb{A} = \langle A_1, \dots, A_n \rangle$ of instrument theories with a positive probability. The uncertainty regarding the phenomena is a function of the shape of the probability distribution on \mathbb{T} and, similarly, the uncertainty regarding the apparatus is a function of the shape of the probability distribution on \mathbb{A} . Information drawn from an experiment changed these shapes, through conditionalization. An experiment is, in fact, a third partition $\mathbb{O} = \langle o_1, \dots, o_k \rangle$, an exhaustive list of observations, one of which will be made. We are interested in the idea that a particular experiment is more a test of the phenomena theory than of the instrument theory, and in the question what strategies are available to bolster the apparatus theory to the point that the apparatus can be used in testing phenomena theory.

An often used measure for the information about the partition T that is contained in the probability function P is

$$I(\mathbb{T}) = \sum_{i=1}^m P(T_i) \log P(T_i)$$

where a term vanishes if $P(T_i)$ is zero (Lindley 1956). This measure reaches a minimum, for fixed m , if P is uniform on the partition, and a maximum, of zero, if $P(T_i) = 1$ for some T_i . After observing o_j , the new probability function $P'(-)$ is $P(-|o_j)$, and thus

$$I(\mathbb{T}|o_j) = \sum_{i=1}^m P(T_i|o_j) \log P(T_i|o_j)$$

Before performing the experiment \mathbb{O} one expects the posterior distribution over T to contain the information

$$EI(\mathbb{T}|\mathbb{O}) = \sum_{j=1}^k P(o_j) I(\mathbb{T}|o_j)$$

And thus the information contained in the experiment \mathbb{O} about \mathbb{T} is $\text{Inf}(\mathbb{O}, \mathbb{T}) = EI(\mathbb{T}|\mathbb{O}) - I(\mathbb{T})$. [One can prove that $\text{Inf}(\mathbb{O}, \mathbb{T})$ cannot be negative.] We can now say that an experiment \mathbb{O} is more a test of the phenomena partition \mathbb{T} than of the apparatus partition \mathbb{A} if $\text{Inf}(\mathbb{O}, \mathbb{T}) > \text{Inf}(\mathbb{O}, \mathbb{A})$.

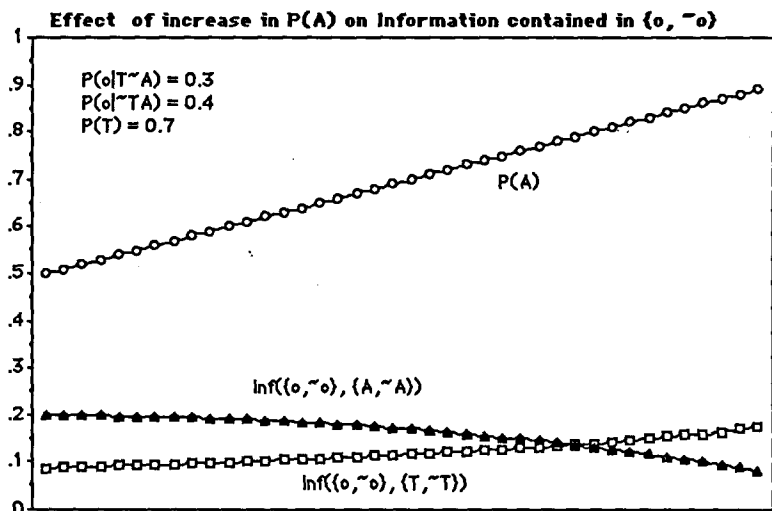
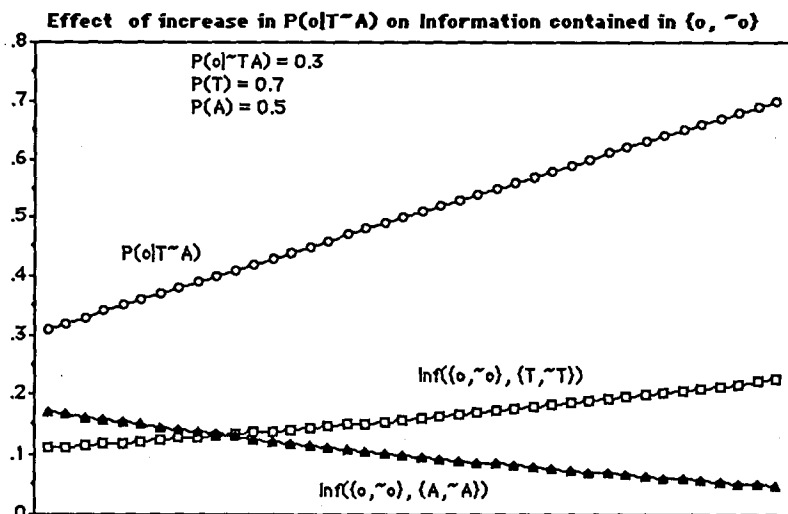


Figure 3 shows the effect of increasing the probability of A (using other evidence vis-à-vis another theoretical partition), one member of the partition $\{A, \sim A\}$ on the information contained in the experiment $\{o, \sim o\}$ regarding the apparatus theory $\{A, \sim A\}$ and the phenomena theory $\{T, \sim T\}$: the experiment slowly turn into a test of the phenomena theory rather than the apparatus theory. Allan Franklin and Colin Howson have discussed a number of strategies to distinguish between valid observations or measurements and artifacts created by the apparatus. Some of these increase the probability of one particular



theory of the apparatus. Others eliminate particular theories of the apparatus that would have made the same prediction in the context of the theory T, as in figure 4.

Above I expressed the suspicion that the discussion of Jon Dorling of historical cases where refuted predictions were blamed on the apparatus rather than on the theory of the phenomena under test, involved experiments that were, even before the data were in, more tests of the apparatus theory than of the phenomena theory (Dorling 1979). This suspicion is formally confirmed by the contrast I have defined here between an experiment that is more a test of the theory of the phenomena than of the apparatus theory. A calculation based on his analysis of Prout's hypothesis show that the experiment to measure the atomic weight of oxygen contained $\text{Inf} = 0.078221$ about Prout's hypothesis and $\text{Inf} = 0.411591$ about the uncertainties regarding the instrumentation involved in the experiment. These experiments, were, as tests of Prout's hypothesis, badly designed.

5. Robert Ackermann's Independence Thesis

In his 1985 book *Data, Instruments, and Theory* Robert Ackermann argued that, for a proper understanding of the growth of science, and of the rationality of its growth, scientific instruments should be brought center stage. He has advanced these two theses concerning apparatus: (i) scientific instruments break the connection between theory and observation so that data can be gathered independent of current theorizing; (ii) instruments tend to break off the influence of assumption on personal observation and make shared data possible. I agree with the second thesis. The increased use of "smart" instruments that do not require the continuous operation of a human observer for the preparation of the instrument and the noticing of the data has indeed led to what I have called elsewhere "observation without an observing subject." (Swijtink 1987). Indeed, as Charles Babbage has said, in his *Reflections on the Decline of Science in England*, "He who can see portions of matter beyond the ken of the rest of his species, confers on obligation on them, by recording what he sees; but their knowledge depends both on his testimony and on his judgment. He who contrives a method of rendering such atoms visible to ordinary observers, communicates to mankind an instrument of discovery, and stamps his own observations with a character, alike independent of testimony and of judgment" (Babbage 1830, p. 169).

But I believe that Ackermann's first thesis needs important qualifications. In the first place, as he himself acknowledges, meters readings have no meaning without a theory of the apparatus A. Thus it is only to the extent that the theory of the phenomena T is independent of the theory of the apparatus A that "data can be gathered independent of current theorizing;" and we need a more detailed map of the extend to which these two types of theory are independent in various sciences.

But Ackermann undoubtedly trows the net of his first thesis in a different direction. It may be argued that, although the "meaning" of o, i.e. e, depends on a theory of the apparatus, the apparatus will function the same, and produce the same o's, when the theory of the apparatus changes. So that, although these o's have no interpersonal shared linguistic description, they can be shared through a common material culture. This must be what Ackermann has in mind when he writes: "Instruments create an invariant relationship between their operations and the world, at least when we abstract from the expertise involved in their correct use. (...) After a change in theory, [the instrument] will continue to show the same reading, even though we may take the reading to be no longer important, or to tell us something other than what we had thought originally" (Ackermann 1985, p.33). In the following I will point at a class of apparatus for which this is not true anymore. These are instruments with aspects of the theory T build-in as assumptions of a computer generated image enhancement technique.

The theory of the phenomena *T* and the theory of the apparatus *A* are intertwined. In the last section of this paper the epistemological complexities of these instruments will be clarified through a Bayesian analysis.

6. Low-dose electron microscopy

What techniques are available to ensure that an instrument satisfies the faithful measuring postulate? The case of low-dose electron microscopy is instructive for the changing relations between theory and instrumentation in the new generation of computer assisted instruments (Abelson 1986). Low-dose electron microscopy is used in the imaging of macromolecules as proteins and ribosomes since high dose radiation would cause damage to the specimen and thus give too many artifacts. But, as Glaeser has shown, there is a significance difference between the minimum number of electrons needed for an interpretable image of a biological object at high resolution (i.e. in the range of 0.2 to 5 nm) and the number of electrons that will cause damage to important details (Glaeser, 1975). But opting for a safe dose of as low as a few electrons per nm², there is a dominating quantum ('shot') noise in the primary image with a very poor signal-to-noise ratio. Thus the observation *o* provided by inspecting a primary image cannot be tied to a reasonably unique measurand *e*, and a low dose electron microscope resulting in a single primary image does not satisfy the faithful measuring postulate.

Furthermore, there is the phase problem. The quantity related to the objects structure is directly proportional to the phase of an electron wave function, since thin specimens of biological material can be characterized by their ability to retard electron that pass through them. The electrons acquire so a phase shift in different proportions. But the phase information is not recorded in a single exposure, since only intensities, i.e. the squared modulus of the wave function, can be recorded. Since the specimen is almost completely transparent for electrons, no contrast is observed.

However, some information is present in each single exposure, especially because of the interference caused by the combined effect of focus, spherical aberration, and electrostatic potential of the specimen. Using several exposures taken with different settings of the defocus parameter of the microscope, computer processing can enhance an image that is informative to the trained eye.

The important epistemological point is that, in the case of non periodic specimens, *apriori* assumptions about the structure of the specimen may have to be presupposed in the enhancement technique (Slump 1984). This example is in contrast to Ackermann's claim that after a change in theory, even in the theory of the apparatus our instruments still behave the same. Theory about the measurand is build right into the smart instrument; without such theory the equipment would not count as an instrument since it would not satisfy the faithful measuring postulate. Naturally, these theoretical assumptions about the measurand should be validated on the basis of other kind of information (e.g. chemical) for the measuring procedure not to be completely circular.

7. A Bayesian analysis of low dose electron microscopy

The type of low dose electron microscopy I am considering here has a stochastic theory. A protein with (unknown) spatial structure *e* is examined through chemical analysis and a prior probability distribution *P** over a set of possible spatial structures *e'* is made an integral part of the computerized enhancement technique. *P** should faithfully represent some reasonable prior over the set of possible spacial structures given the background knowledge and has a description in the apparatus theory *A*. A series of primary electron microscopy exposures of the protein is combined with the

prior distribution P^* and, ideally, converges on an image o which is presented to a trained observer. Thus

$$P(o|P^*, e) = 1 \text{ for some image } o$$

The observer interprets image o as spatial structure through the judgment " $I(o) = e'$ ", where e' may be different from e . We will assume that this interpretation process is deterministic, shared by all trained observers, and independent of e and P^* , since the instrument may be a black-box or off-the-shelf device for the observer:

$$P(I(o) = e'|o, P^*, e) = P(I(o) = e'|o) = 1 \text{ for some structure } e'$$

We expect that the convergence process and imaging technique are such that, if $P^*(e)$ is not too small, in the limit only correct guesses will be made:

$$P(I(o) = e|P(o|P^*, e) = 1, P^*, e) = 1$$

But the convergence to the truth may not be fast enough, or P^* may not cover the truth, and false calls will be unavoidable.

Thus $P(o|P^*, e)$ will generally not be identical one for some image o and zero everywhere else. Let α_{ee} be

$$\alpha_{ee} = \sum_o P(o|P^*, T, e) \text{ where } I(o) = e$$

i.e. α_{ee} is the probability of making a correct guess when P^* is the adopted measure in the image enhancement program, T the correct theory, and e the structure of the specimen. We assume that $\alpha_{ee} = \alpha$, that is, the same for each specimen. Since we still assume that

$$P(I(o) = e'|o) = 1 \text{ for some structure } e'$$

we now have

$$\sum_o P(o|P^*, T, e) = \alpha \text{ where } I(o) = e$$

Let $\alpha_{e'e}$ be

$$\sum_o P(o|P^*, T, e) \text{ where } I(o) = e'$$

$$P(I(o) = e'|o) = \alpha_{e'e} \text{ where } I(o) = e'$$

$\alpha_{e'e}$ may be a function of the 'distance' between e' and e which suggests $\alpha_{e'e} = \alpha_{ee'}$. We are again interested in the kind of support a theory can obtain from judgments made by trained observers using such an instrument. Let T and T' be two theories of the spatial structure of proteins, and let them predict that a certain protein has structure e or e' , respectively. Let P^* be determined and the enhancement process lead to image o which is then interpreted as $I(o)$. What is

$$\frac{P(T|I(o))}{P(T'|I(o))} ?$$

$$\text{By Bayes's theorem: } \frac{P(T|I(o))}{P(T'|I(o))} = \frac{P(I(o)|T) \cdot P(T)}{P(I(o)|T') \cdot P(T')}$$

where

$$P(I(o)|T) = \sum_{o': I(o') = I(o)} P(I(o)|o') P(o'|e, T, P^*) \cdot P(P^*|T) =$$

$$\sum_{o': I(o') = I(o)} P(I(o)|o') P(o'|e, T, P^*)$$

since P^* is determined by background knowledge and e is implied by T . The important point is to judge how dependent $P(o|e, P^*, T)$ is on the theory of the phenomena. Does the actual structure of the protein e screen off the theory T ? This will depend on the nature of T . If T is a rather straightforward classification and systematization of spatial structures, it seems reasonable to assume that $P(I(o)|P^*, T, e)$. On the other hand, if T embeds its classification and systematization of spatial structure in a biochemical, explanatory account that touches on the permeability of proteins by electrons, independence may fail.

Proceeding on the assumption that the rates for errors and correct judgments are, given the background knowledge, independent of the theory of the phenomena we get

$$P(I(o)|T) = \sum_{o^*: I(o^*) = I(o)} P(I(o)|o^*) \cdot P(o^*|e, P^*) \text{ and thus}$$

$$\frac{P(T|I(o))}{P(T'|I(o))} = \frac{\alpha_{eI(o)}}{\alpha_{e'I(o)}} \cdot \frac{P(T)}{P(T')}$$

Thus, even though T and T' are deterministic theories that make different predictions and even though the theory of the apparatus A is assumed known, even if $I(o) = e'$, $P(T|I(o))$ may still be positive and

$$\frac{P(T|I(o))}{P(T'|I(o))}$$

may still be > 1 . The faithful measurement postulate only requires that $\alpha = \max\{a_{e'e}\}$ since then the observer's judgment will converge towards the truth.

8. Conclusion

The low dose electron microscope is an exception to Robert Ackermann's thesis that after a change in theory an instrument will continue to show the same reading. It is one of a growing class of 'smart' instruments in which the faithful measurement postulate is enforced through theory dependent adjustment on an incoming signal. Here I showed that inverse inference is still possible for these types of instruments.

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