ARTIFICIAL AND NATURAL NEAR-INFRARED RADIATION EFFECTS UPON THE CELL MOTILITY. BIOLOGICAL HYPOTHESE

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ABSTRACT

We proved that the centrioles were the device used for the detection of an artificial infrared source external to 3T3 fibroblast and epithelial CV1 cells. The fundamental contribution of the cytoskeleton explains the cell motility. In effect the strain due to the physical pressure on the plasma cell membrane is a sufficient stress to activate Hsp 27 which results in actin polymerization and motility of the cell. We calculate the diffracted field inside the cell due to the current induced along the centriole equivalent to a cylindrical dipole. We deduced the centriole bistatic area function of the directions of the incident and diffracted fields. Some idea of the theoretical pressure equal to several $nN/\mu m^2$ is in good agreement with recent experiments. About the two centrioles in orthogonal positions 100 % of the space is covered, while during the experiments 88 % of 3T3 and 50 % of CV1 cells were put in motion in presence of a near infrared source. So we must consider than the strain due to the pressure on the membrane can be transmitted to the side facing the light source throughout the actin cyto-skeleton. The stress fibers serve as force transmitters in fast mecanotransduction and act as mechano-sensors with direction sensitivity on slow mechanotransduction. We determine the spectral emission power of a single cell from the black-body one. Finally a cell scatters a weak ambient black-body theoretical power radiation. The cellular agregate radiation is able to be detected by one cell located at a certain distance following recent measurements.

INTRODUCTION

Albrecht-Buehler showed that 3T3 mouse fibroblast cells and epithelial CV1 cells could react to a near-infrared light by moving either towards or away from the light source. The type of response depended on the cell type and on the temporal pattern of the emission of the light. He also proved that the centrioles were the detection device for the direction of the infrared source [1] - [4].

CONTRIBUTION OF THE CYTOSKELETON CONCERNING THE CELL MOTILITY (fig. 1)

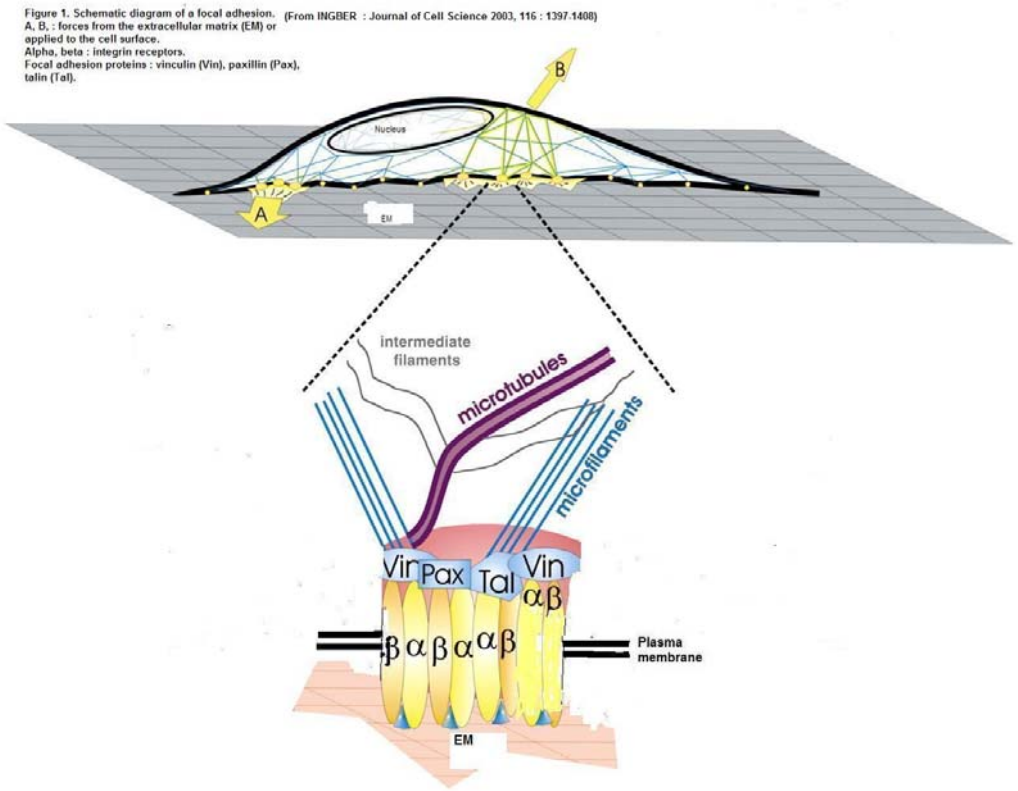

Fig. 1 : Schematic diagram of a focal adhesion.
(From INGBER : Journal of Cell Science 2003, 116 : 1397-1408)
A, B : forces from the extracellular matrix (EM) or applied to the cell surface
Alpha, beta : integrin receptors.
Focal adhesion proteins : vinculin (Vin), paxillin (Pax), talin (Tal).

The plasma membrane is an assembling of various proteins inserted in the fluid matrix of a lipid bilayer. In non muscle cells integrins receptors are membrane spanning proteins that ligate extracellular matrix proteins and link to actin skeleton on the inside of the cells [5] providing a great degree of mechanical coupling across the cell surface. The cytoskeleton is composed of microtubules, actin microfilaments and intermediate filaments. Application of forces to integrins receptors and associated focal adhesion proteins results in physical distorsion of the membrane surface and repositionning of the cytoskeletal filaments along applied field lines within the cytoplasm [6]. Among the three filament systems that form the cytoskeleton, the actin network plays the principal role in determining the cell dynamic response [7]. The cell motility is driven by the sum of asymmetric traction forces exerted on the substrate through adhesion foci that interface with the actin skeleton [8] stimulating polymerization of monomeric globular actin to filamentous actin [9]. Polymerization of actin filaments is necessary for the extension of membrane structures, creating a propulsive force that pushes forward the lamellipodia at the front of migrating cell [10], [11]. Stress proteins, called heat shock proteins (Hsps), are synthesized by cells in case of stress. In the small heat shock protein family, Hsp27 participed in regulating the organization of the actin cytoskeleton in both control and stress conditions. When Hsp27 is phosphorylated, its stimulates actin polymerization and modulates the rate of polymerization, therefore determining the motility of the cell [10], [12], [13], [14], [15]. So we could assume that the strain due to the pressure on the plasma

membrane, resulting from the near infrared radiation, constituted a sufficient stress to activate Hsp27 which resulted in actin polymerization and motility of the cell.

INFLUENCE OF A FLUCTUATING NEAR-INFRARED EXTERNAL SOURCE ABOUT THE CENTRIOLE DIFFRACTION INSIDE THE CELL

Albrecht-Buehler G. showed [1], [4], that a pair of centrioles is able to detect the direction of a near-infrared pulsed source of a 0.8 µm wavelength. Recently [16] we introduced the theoretical selective transparence of the cell membrane deduced from experimental results in the range stretched from 0.2 to 1µm. It was called the transmission coefficient T_{13} . Then from an isotropic radiated power of 4 µW of the light source located at 60 µm, we calculated, inside the cell, an electric field E_i equal to 40 V/m. The wave attenuation along the path and the transmission through the membrane (T_{13}) were taken into account. Inside the cell the mean incident power per unit of area is equal to :

$$p_i = \frac{1}{2}\sqrt{\frac{\varepsilon_o}{\mu_o}}\left|\vec{E}_i\right|^2 \qquad (1)$$

We compare each protofilament of the centriole to a cylindrical dipole of length 2h and diameter 2a, which is equivalent to an opened transmission line of characteristic impedance Z_c and linear propagation constant : $\gamma = \alpha + jk$ [17].
We use the following parameters without dimension :

$$\left.\begin{aligned} K = kh = 2\pi h/\lambda, \qquad \Omega = 2\log_e(2h/a), \\ A = \alpha h = (kh)^2 \big/ 2(\Omega - 3.4) \end{aligned}\right\} \qquad (2)$$

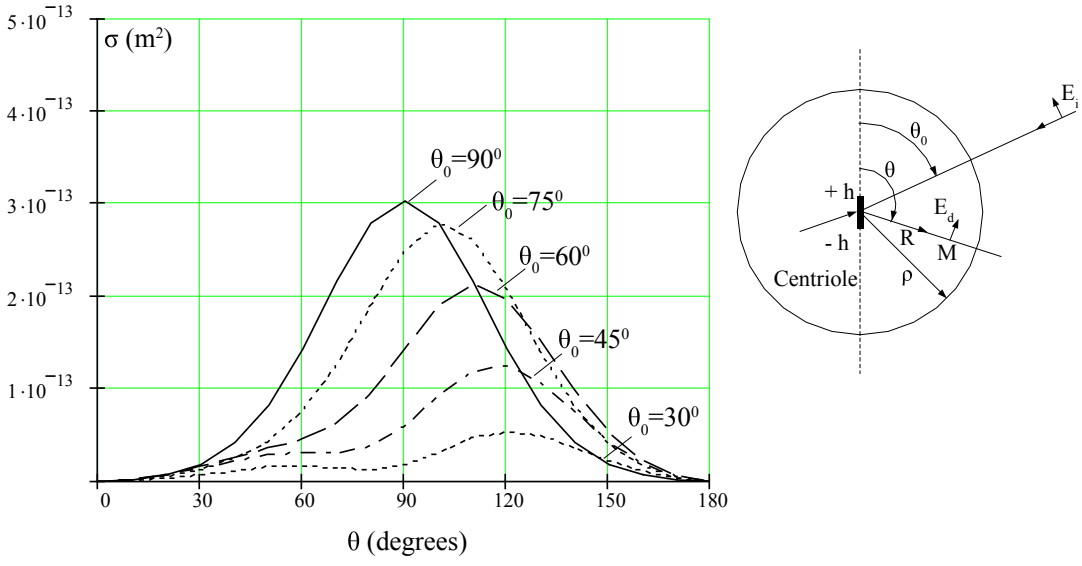

Fig. 2. Cell bistatic area σ (θ, θ_0)

The figure 2 shows the centriole lighted by a plane-wave from the θ_o direction with an electric field $\vec{E}_i(\theta_o)$. The diffracted field at point M : $\vec{E}_d(\theta,\phi,\theta_o,R)$ $(0 \le \phi \le 2\pi)$, inside the cell is due to the current $I(z,\theta_o)$ induced along the centriole. The distance between the center of the centriole and M is R. The bistatic area of the centriole related to the two directions θ_o and θ is given by the following expression :

$$\sigma(\theta,\theta_o) = \frac{\lambda^2}{\pi}\left|S(\theta,\theta_o)\right|^2 \qquad (3)$$

$$\text{with :} \quad \left|\frac{\vec{E}_d(\theta,\theta_o,R)}{\vec{E}_i(\theta_o)}\right|^2 = \frac{\left|S(\theta,\theta_o)\right|^2}{(kR)^2} = \frac{\sigma(\theta,\theta_o)}{4\pi R^2} \qquad (4)$$

The expressions (3) and (4) are valid when $R \ge 8h^2/\lambda$ (5), that is when the diffracted field is associated with an electromagnetic plane-wave.

$I(z,\theta_o)$ and $S(\theta,\theta_o)$, which depend on A, K and Ω parameters are given in the annex of calculation.
The whole power P_d diffracted inside the cell by the centriole is :

$$P_d(\theta_o) = \frac{1}{2}\sqrt{\frac{\varepsilon_o}{\mu_o}}\int\left|\vec{E}_d\right|^2 ds = \frac{1}{2}\sqrt{\frac{\varepsilon_o}{\mu_o}}\left|\vec{E}_i\right|^2 \int\frac{\sigma(\theta,\theta_o)\,ds}{4\pi R^2} = \frac{p_i}{2}\int_o^{\pi}\sigma(\theta,\theta_o)\sin\theta\,d\theta \qquad (6)$$

with $ds = R^2 \sin\theta\, d\theta\, d\phi$.

For the pulse light emission the form factor is the product of the length of time τ by the frequency f_r.
In a corresponding stochastic way the energy is stored inside the cell of radius ρ, where it is trapped because its transmission through the membrane is lower than 10 percent [16].

The stored energy during a length of time Δt will be equal to :

$$W_S = P_d(\theta_o)\,\Delta t\,\tau\,f_r \qquad (7)$$

The density of energy u_S and the mean pressure P applied on the membrane are then :

$$u_S = \frac{W_S}{(4/3)\pi\rho^3} \qquad (8) \qquad\qquad P = \frac{u_S}{3} \qquad (9)$$

In the fig. 2 we present the bistatic area (3) in terms of θ for several θ_o values with the following parameters given in (2) :

$$2h = \lambda = 0.8\,\mu m, \quad 2a = 40\,nm, \quad \Omega = 7.38, \quad K = \pi, \quad A = 1.24$$

The validity condition (5) is fulfiled when : $R \ge 1.6\,\mu m$. Whatever, the chosen incident angle θ_o, the max of σ and P_d occur for $\theta \ge 90°$. The active diffracted beam draws an asymmetrical cone-shaped zone around the centriole axis so that the diffracted light propagation goes away from the light source.
The Table I shows P_d, W_S, u_S and P with $p_i = 2W/m^2$ (1), $\tau f_r = 1/2$,

$\rho = 11\,\mu m$ and $\Delta t = 600\,s$.

θ_o (degrees)	90	75	60	45	30
P_d (watts) (6)	3.10^{-13}	3.10^{-13}	$2.6\,10^{-13}$	$1.4\,10^{-13}$	$7\,10^{-14}$
W_S (Joules) (7)	9.10^{-11}	9.10^{-11}	8.10^{-11}	4.10^{-11}	2.10^{-11}
u_S (Joules/m^3) (8)	$1.6\,10^4$	$1.6\,10^4$	$1.4\,10^4$	$7.4\,10^3$	$4\,10^3$
P(nN/μm^2)	5.0	5.0	4.6	2.3	1.2

Table I : Power and energy diffracted by a 3T3 cell.
Pressure applied on the membrane

Some idea of the size of the pressure P has been obtained in recent experiments [18]. In the abstract we can read: "we measure dynamic traction forces applied by epithelial cells (monolayer) on a substrate. The force sensor is a high-density array of elastomeric microfabricated pillars that supports the cells. Traction forces induced by cell migration are deduced from the measurements of the bending of these pillars and are correlated with actin localization by fluorescence microscopy". Each post or pillar is a cylinder of radius r and length L. The deflection Δx of the free end of the post is due to a force F exerted by the cell on its underlying substrate given by :

$$F = \left(\frac{3}{4}\pi E \frac{r^4}{L^3}\right)\Delta x \qquad (10)$$

E is the Young's modulus of the pillar.

With $\Delta x = 0.1\,\mu m$, $r = 1.5\ \mu m$, $L = 5\ \mu m$, $E = 2\ 10^6\ P_a$, we find : F = 19 nN.

This force applied to the area of the pillar $\pi r^2 = 7.07\ \mu m^2$ corresponds to a pressure of 2.7 nN/μm^2.
This pressure is in good agreement with our theoretical results shown in Table I.
Previous analysis (2001, 1999), related in [18], have indicated a 5.5 nN/μm^2 value relating force and contact area.

Note : Albrecht-Buehler said (April 23, 2003) : *"The cells used were always moving. The first sign of changing direction, i.e the extension of a new lamellipodium in the direction of the light source, was usually visible after 5-10 min (factor 2) since the beginning of the lighting"*. This explains the choice of $\Delta t = 600$ s in W_S (7).

Then the radiation by one centriole is the most efficient when $45° \le \theta_o \le 135°$ which correspond to a diffraction zone : $60° \le \theta \le 120°$ (fig 2) for a variation of σ and P_d by a same factor 2. If the two centrioles are in orthogonal positions 100 % of the space is covered. According to Albrecht-Buehler about 88 % of 3T3 cells and 50 % of CV1 cells were put in motion in presence of near-infrared source. This information is related in [19].

CELL SCATTERING OF A NEAR-INFRARED FRACTION OF THE BLACK-BODY RADIATION

Recently Albrecht-Buehler G. explains that a cell can scatter the near-infrared portion of the ambiant black-body radiation to become a light source, as a result of the experiments [20]. The present purpose is to justify a

such hypothesis giving some theoretical development. We are sure that the scattered light of the black-body radiation for one cell in the near-infrared range is very small and cannot be detected, but may be by a group of aggregated cells.

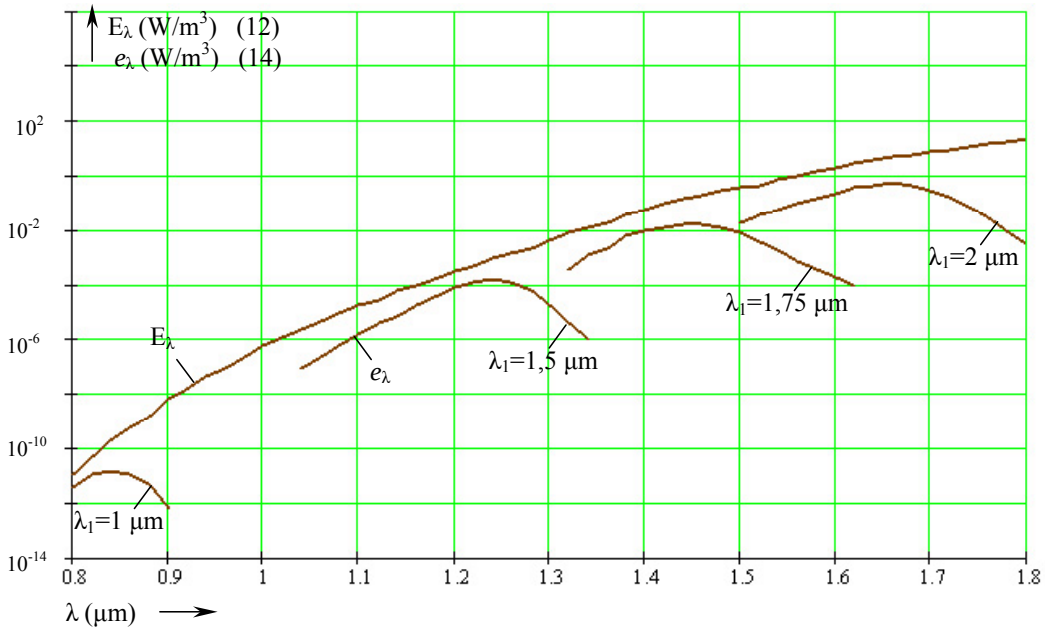

Fig. 3. Spectral emission powers E_λ and e_λ (for T=310°K).

The spectral-emission power E_λ is given by :

$$E_\lambda = \frac{2hc^2}{\lambda^5}.\frac{1}{\exp\left(\frac{h\nu}{kT}\right)-1} \qquad (11)$$

In international units it is expressed in W/m^3 :

$$E_\lambda = \frac{1.19\,10^{-16}}{\lambda^5}.\frac{1}{\exp\left(\frac{14.4\,10^{-3}}{\lambda T}\right)-1} \qquad (12)$$

The whole specific intensity E_T of the black-body expressed in W/m^2 is :

$$E_T(\lambda,T) = \int_0^\infty E_\lambda \, d\lambda \qquad (13)$$

The spectral-emission power of one cell is equal to :

$$e_\lambda = \alpha_\lambda E_\lambda = |T_{13}|^2 . E_\lambda \qquad (14)$$

$\alpha_\lambda < 1$ is the absorption coefficient, and T_{13} the transmission coefficient through the cell membrane given in [16] by :

$$|T_{13}|^2 = 1 \Big/ \left[ch^2(\Gamma_2 d) + k_2^2 sh^2(\Gamma_2 d) \Big/ 4n_1^2 \right] \qquad (15)$$

d is the membrane thickness and $\Gamma_2 d = 2\pi k_2 d/\lambda$. k_2 is the index of absorption of the membrane and n_1 is the index of refraction of the interstitial medium. We present in fig. 3 the spectral-emission powers E_λ and e_λ in terms of λ for T = 310° K. The parameter λ_1 is the maximal wavelength which limits the near-infrared absorption range. The max of E_λ is equal to $10^7\,W/m^3$ for $\lambda = 9.3\,\mu m$.
The whole specific intensity e_T of the cell is then given by :

$$e_T = \int_0^{\lambda_1} e_\lambda d\lambda \qquad (16)$$

It appears in the table II :

$\lambda_1\,(\mu m)$	1	1.5	1.75	2
$e_T\left(\mu W/cm^2\right)$ (16)	10^{-16}	10^{-9}	2.10^{-7}	8.10^{-6}

Table II (valid for T = 310 °K)

According to the second principle of thermodynamics, at $T = 310°K$, the power radiated by a cell of radius ρ is : $P_d = 4\pi\rho^2\, e_T$ (17)

For a 3T3 cell with : $\rho = 11\,\mu m$, P_d is shown in Table III.

$\lambda_1\,(\mu m)$	1	1.5	1.75	2
P_d (watts) (17)	$1.5\,10^{-27}$	$1.5\,10^{-20}$	3.10^{-18}	$1.2\,10^{-16}$

Table III – Power radiation by a 3T3 cell

We note the great importance of the limit of the membrane cell transparence upon its natural radiation power. So a cell scatters a weak ambient black-body radiation. Only aggregate cells is able to be detected by one cell located at a certain distance as recently measured [20].

BIOLOGICAL HYPOTHESE

Could the pressure P calculated in (9) be the cause of the displacement of the cell to protect the overall integrity of the membrane ?

The strain (fig. 1, A) due to the pressure P, is received in focal adhesion proteins and integrins activate the signaling pathway leading to actin polymerization and motility of the cell towards the light source. However Albrecht-Buehler observed more cells were going forward than backward. So we must consider that the strain on the membrane can be transmitted to the side facing the light source. Externally applied forces (fig. 1, A) are transmitted throughout the actin cytoskeleton and result in immediate repositionning of cytoskeletal filaments along applied tension field lines within the cytoplasm [6], [21]. Stress fibers, composed of actin filaments, myosin and α-actinin, are anchored at focal adhesions at one side of the cell, and at focal adhesions on the other side of the cell. They serve as force transmitters in fast mechanotransduction and act as mechanosensors with direction sensitivity on slow mechanotransduction [22]. So the strain due to the pressure could be transmitted to the opposite side of the cell where it would determine the moving of the cell away the light source. This can be akin to the transmission of tension due to fluid shear stress from the apical surface of endothelial cells through the cytoskeleton to the integrins which then change in tension (23). Moreover, in the Albrecht-Buehler's experiments, the percentages of cells that were either attracted or pushed away from the light source depended on the pulsing frequencies of the light source. Were the threshold between a fast and a slow mechanotransduction linked to the difference in frequency ?

SUMMARY

The 3T3 fibroblast and epithelial CV1 cells motility has been explained :

- firstly by their centrioles diffraction under the influence of a fluctuating near-infrared artificial external pulsed source. The diffraction light propagation is such that, due to the physical pressure on the plasma cell membrane, the cell goes towards the light source.
- secondly when a sufficient strain, transmitted along the cytoskeleton, can move the cell away the light pulsed source.

On the other hand, we show that a cell scatters a weak part of the natural near infrared ambient black body radiation.
All these theoretical results are in good agreement with recent published measurements.

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APPENDIX

Induced current along the centriole due to the incident electric field $\vec{E}_i(\theta_o)$ (figure 1)

It is given by the following equation [17] :

$$I(z,\theta_o) = C\left\{-j\frac{\sin(K\cos\theta_o)}{sh(A+jK)}sh\left[\frac{z}{h}(A+jK)\right]-\right.$$

$$\left.-\frac{\cos(K\cos\theta_o)}{ch(A+jK)}ch\left[\frac{z}{h}(A+jK)\right]+\exp(jKz\cos\theta_o)\right\}$$

$$\text{with :}\quad C=\frac{2hE_i}{Z_c}\frac{(A+jK)\sin\theta_o}{\left[(A+jK)^2+K^2\cos^2\theta_o\right]},\qquad Z_c=\frac{Z_o}{2\pi}\left(1-j\,2\frac{A}{K}\right)^{1/2}(\Omega-3,4)$$

With the previous parameters, $E_i=40\,V/m,$ and $Z_o=377$ ohms, the module of the current $\frac{2\,h\,E_i}{Z_c}$ is equal to 0.12 μA.

Bistatic area of the centriole

The function $S(\theta,\theta_o)$ is extracted from [17] : $S(\theta,\theta_o)=j\frac{K\,Z_o}{2\pi Z_c}F(\theta,\theta_o)$

$$\text{with}\qquad F(\theta,\theta_o)=(p+q+r).\frac{s}{u}$$

$$\text{and}\quad p=K\frac{shx}{x}\left[\frac{-z}{ch(A+jK)}+j\frac{t}{sh(A+jK)}\right]\qquad q=-\left(K\frac{sh\,y}{y}\right)\left[\frac{z}{ch(A+jK)}+j\frac{t}{sh(A+jK)}\right]$$

$$r=2\frac{\sin(Kv)}{v}\qquad s=(A+jK)\sin\theta\sin\theta_o$$

$$u=(A+jK)^2+K^2\cos^2\theta_o\qquad x=A+jK(1-\cos\theta)$$

$$y=A+jK(1+\cos\theta),\qquad z=\cos(K\cos\theta_o),\qquad t=\sin(K\cos\theta_o),\qquad v=\cos\theta+\cos\theta_o$$